obtained under Rous LTR promoter control and the unusual transformation competence of CV-1 cells. Levels of vector expression immediately after transfection, perhaps reflecting the number of transcriptionally active templates, may determine the probability of a productive stable integration event occurring. Consistent with this hypothesis is the observation that treatment of CV-1 cells with sodium butyrate immediately after transfection results in five- to tenfold increases in both transient expression (assayed with pSV2cat) and stable transformation (assayed with pSV2gpt) (*13*).

The proportion of CV-1 cells that transiently express exogenous vector DNA and then become stably transformed appears to be high. In earlier experiments we used immunofluorescent labeling with CAT antibody to show that, with our standard DNA-mediated transfection conditions, about 10 percent of CV-1 cells transiently express pRSVcat (*4*). Since at least 5 percent of CV-1 cells form colonies after transfection with pRSVgpt under the same conditions, more than half the cells that exhibit transient expression appear to become stably transformed. The number of integrated gpt copies in each of six individual clones examined varied between 1 and 10 (data not shown). No evidence has been found suggesting that either pSV2gpt or pRSVgpt replicates as an episomal plasmid in these cells (*4, 5*).

Although integration of plasmid DNA apparently occurs in the majority of CV-1 cells, our data do not necessarily imply that these cells are exceptionally proficient at integration of exogenous DNA. The overall transformation efficiency may reflect rather the relative stability of integrated copies. Supporting this idea is the finding that neither pSV2gpt$^-$ nor pRSVgpt$^-$ CV-1 colonies appear to segregate at high frequency as do many early mouse L cell herpes tk transformants (*14*).

We did not investigate stable expression of nonselected genes in this study; however, it has been shown that about 25 percent of CV-1 clones isolated after cotransfection with unligated pSV2gpt and pSV2neo vectors express both markers (*6*). This cotransformation frequency, combined with the unusually high competence of CV-1 cells for DNA-mediated transfection, suggests that these cells should find application in screening genomic DNA or eukaryotic complementary DNA (cDNA) expression vector libraries.

Our results as well as our experience with additional cell lines indicate that, for each cell type, it is important both to select a vector with an appropriate promoter and selectable marker combination and to optimize parameters for DNA-mediated transfection (for example, by using the CAT assay). With this approach, it should be possible to stably transform many continuous monolayer cell lines with efficiencies of 10^{-3} (0.1 percent) or higher. Nonimmortalized "primary" cells appear to be less competent recipients for DNA-mediated gene transfer under conditions that we have examined so far. Nevertheless, we find that WI38 human embryo fibroblasts may be stably transformed with pRSVneo at a frequency of at least 10^{-5} (*15*).

CORNELIA GORMAN
RAJI PADMANABHAN
BRUCE H. HOWARD

Laboratory of Molecular Biology, Division of Cancer Biology and Diagnosis, National Cancer Institute, Bethesda, Maryland 20205

References and Notes

1. For reviews, see G. M. Cooper, *Science* **217**, 801 (1982); R. A. Weinberg, *Cell* **30**, 3 (1982); J. M. Bishop, *ibid.* **32**, 1018 (1983).
2. K. Shimizu *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 2112 (1983).
3. C. M. Gorman, L. F. Moffat, B. H. Howard, *Mol. Cell. Biol.* **2**, 1044 (1982).
4. C. M. Gorman, G. T. Merlino, M. C. Willingham, I. Pastan, B. H. Howard, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 6777 (1982).
5. R. C. Mulligan and P. Berg, *ibid.* **78**, 2072 (1981).
6. P. J. Southern and P. Berg, *J. Mol. Appl. Genet.* **1**, 327 (1982).
7. B. H. Howard and P. Berg, unpublished results.
8. SV40-transformed xeroderma pigmentosum cells (XP_2OS-SV40 line) were provided by K. Kraemer (NCI).
9. I. Abraham *et al.*, *Somat. Cell Genet.* **8**, 23 (1982).
10. C. M. Gorman, R. Padmanabhan, B. H. Howard, in *NATO Conference on Genetics in Eukaryotes*, P. Lurquin and A. Kleinhofs, Eds. (Plenum, New York, 1983), p. 61.
11. Plasmid DNA (form I) was prepared from lysozyme-Triton X100–cleared lysates by two banding steps in CsCl and ethidium chloride equilibrium gradients. Cell lines were grown in Dulbecco's minimal essential medium (DMEM) freshly supplemented with 10 percent fetal calf serum (FCS), glutamine (2 m*M*), penicillin (50 unit/ml), and streptomycin (50 μg/ml). For growth of NIH/3T3 cells, 10 percent calf serum was substituted for FCS; for growth of CHO cells alpha MEM was substituted for DMEM. Cells were split every 4 to 5 days with trypsin (0.25 percent), except CV-1 cells, which required treatment with a solution of trypsin (0.2 percent) and EDTA (2 m*M*). The pCO_2 in tissue culture incubators was periodically adjusted to ensure that medium in the absence of cells equilibrated at *p*H 7.3 to 7.4. Transfection competence of our stock of CV-1 cells frequently declined after ten passages, requiring lower passage cells to be thawed from liquid $nitrogen_2$ storage. Treatment of cells after DNA-mediated transfection with 15 percent (weight to volume) glycerol in Hepes buffer saline ("glycerol shock") was 3 minutes for CV-1 cells but was reduced to as short a time as 30 seconds for other cell types to prevent crenation or shrinkage (or both) of cells before being washed with DMEM. The CAT assay may be used to optimize conditions as follows: transfect 5×10^5 CV-1 cells per 10-cm plate with 10 μg of pSV2cat DNA; harvest 14 to 16 hours after glycerol treatment; assay 10 μl of the cellular extract (which was 100 μl) from one plate in a standard reaction (*3*) containing 0.5 μCi of ^{14}C-labeled chloramphenicol (50 mCi/mmole; New England Nuclear). After a 20-minute incubation period about 20 percent of the ^{14}C-labeled chloramphenicol should be converted to acetylated forms. More detailed protocols describing preparation of DNA, as well as maintenance and transfection of tissue culture cells, are available on request.
12. F. L. Graham, S. Bacchetti, R. McKinnon, K. C. Stanners, B. Cordell, H. M. Goodman, in *Introduction of Macromolecules into Viable Mammalian Cells*, R. Baserga, C. Croce, G. Rovera, Eds. (Liss, New York, 1980), pp. 3–25; S. Silverstein, personal communication.
13. C. M. Gorman *et al.*, in preparation.
14. G. A. Scangos, K. M. Huttner, D. K. Juricek, F. H. Ruddle, *Mol. Cell. Biol.* **1**, 111 (1981).
15. R. Padmanabhan, C. Gorman, B. Howard, unpublished results.
16. F. Graham and A. van der Eb, *Virology* **52**, 456 (1973).
17. E. Frost and J. Williams, *ibid.* **91**, 39 (1978).
18. Supported in part by an American Cancer Society postdoctoral fellowship (PF-1924) to C.M.G.

28 March 1983; revised 26 June 1983

Expression of the Major Neurofilament Subunit in Chicken Erythrocytes

Abstract. *The 70,000-dalton core polypeptide of neurofilaments, thought to exist only in neurons, has been detected in chicken erythrocytes, where it coexists with vimentin and synemin as a component of the intermediate filament network. It is present in the circulating erythroid cells of embryos and young chickens but is nearly absent from the erythroid cells of adults. These findings are inconsistent with current models of intermediate filament expression, but provide another example of unexpected similarities between the nervous and hemopoietic systems.*

Intermediate filaments are linear polymers of polypeptides with subunit molecular masses of 40 to 70 kilodaltons (40 to 70K) and diameters of approximately 10 nm. The filaments can be divided into five general classes, on the basis of subunit biochemistry, antigenicity, and cell type distribution: keratin filaments in most epithelia, neurofilaments in neurons, glial filaments in astrocytes, desmin filaments in muscle, and vimentin filaments in mesenchyme derivatives and in many undifferentiated cells and cultured cells [see (*1*) for a review].

Neurofilaments are composed of a 68 to 70K polypeptide (NF70) (*2*) and two larger polypeptides in association with the core (*3*). This triplet of neurofilament polypeptides (*4*) is now thought to exist only in neurons, although a previous suggestion to the contrary (*5*) resulted from confusion of the 70K neurofilament subunit with the 68K "heat shock" protein, which is found constitutively in

most cell types and copurifies in part with many different cellular components, including intermediate filaments (*6, 7*). Here we show conclusively that NF70 is also present in chicken erythrocytes, and that, in contrast to neurons, its erythroid expression diminishes as the chicken approaches adulthood.

Chicken erythrocytes have a cytoplasmic network of intermediate filaments (*8*) composed predominantly of vimentin (*9*); these filaments exhibit a periodic, peripheral association of synemin (*9, 10*), a 230K polypeptide initially shown to be associated with desmin and vimentin filaments in muscle cells (*11*). In erythrocytes, the periodicity of synemin along the core vimentin filament changes during development, decreasing from an average of 230 nm in embryonic cells to 180 nm in adult cells (*10*). An acidic, 70K polypeptide was found to copurify in minor amounts with vimentin and synemin from chicken erythrocytes [figure 2a in (*9*)]; this polypeptide (E70) has an isoelectric point and electrophoretic mobility identical to those of NF70 from chicken nervous tissue (Fig. 1, a to c).

To substantiate the tentative identification of E70 as the neurofilament polypeptide, antibodies for NF70 from chicken spinal cord were prepared in a rabbit. When assayed by immunoautoradiography (*9, 12*) on two-dimensional gels of either whole brain or a spinal cord neurofilament fraction (Fig. 1b), the antiserum specifically labels NF70 (Fig. 1d). Prolonged exposure of the autoradiogram reveals a few faintly labeled spots of lower molecular weight; these are presumed to be proteolytic fragments of NF70 (*9, 11, 13*). Immunoautoradiographic analysis of chick erythrocyte intermediate filaments or membrane fractions (Fig. 1c) demonstrates specific antigenic cross-reactivity of the corresponding erythrocyte 70K polypeptide (E70) with this antiserum (Fig. 1e). Presumptive proteolytic fragments are also evident on overexposure, with a pattern similar to that seen for NF70. Neither vimentin nor synemin is detectably labeled with this antiserum.

Identity of NF70 and E70 was confirmed by two-dimensional chymotryptic peptide mapping. NF70 and E70 were excised from two-dimensional gels similar to those in Fig. 1, b and c, iodine-labeled, digested with α-chymotrypsin, subjected to electrophoresis (left to right), and chromatographed (bottom to top) on thin-layer cellulose plates, and autoradiographed (*14*). The resulting iodopeptide maps of NF70 and E70 are virtually identical (Fig. 1, f and g), indicating highly homologous if not identical

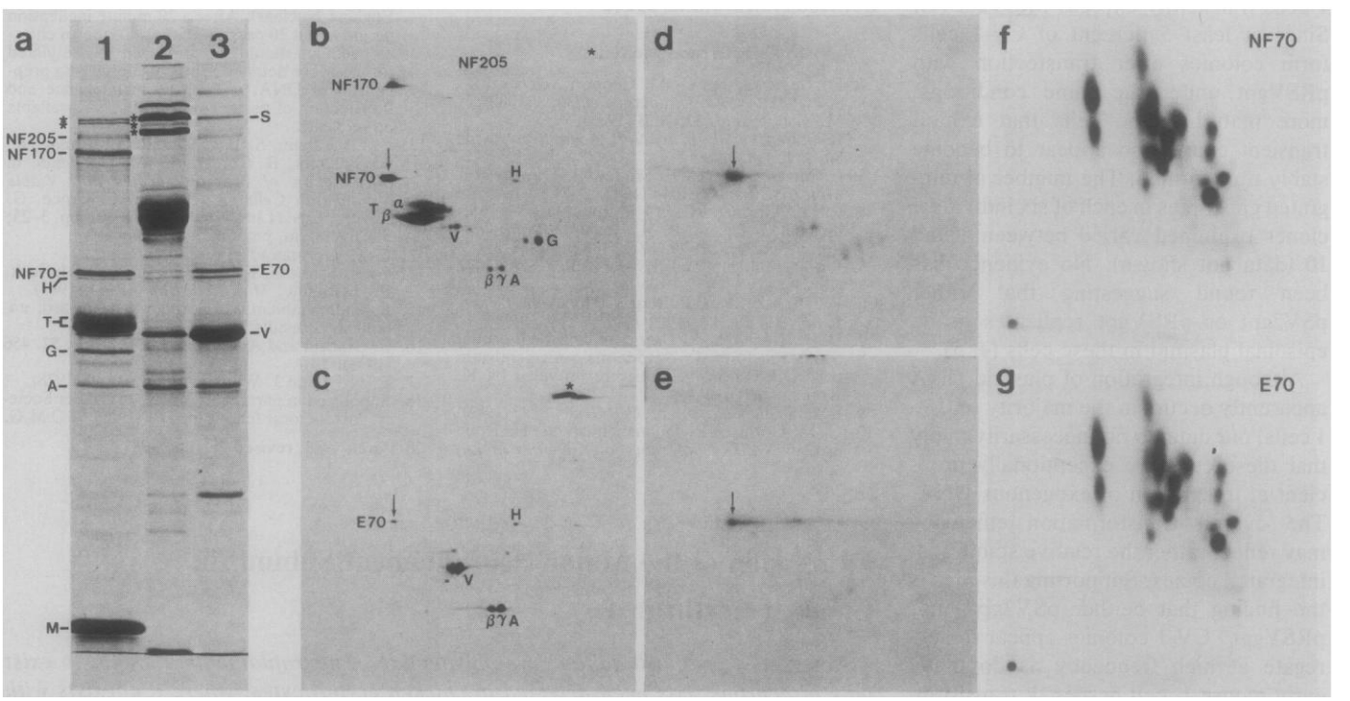

Fig. 1. (a) Sodium dodecyl sulfate polyacrylamide gel (*9, 28*) of a crude neurofilament preparation from chick spinal cord (lane 1), chick erythrocyte plasma membranes to which a portion of intermediate filament network is bound (lane 2), and a low-salt extract of these membranes, enriched for the intermediate filament proteins (*9*) (lane 3). Spinal cords from month-old chickens were homogenized in a glass Dounce homogenizer in a buffer of 5 m*M* Pipes, 5 m*M* EDTA, 1 m*M* NaN_3, and 0.1 m*M* phenylmethylsulfonyl fluoride (PMSF) (*p*H 6.7 at 0°C) and centrifuged at 15,000*g*. The supernatant was made 100 m*M* in NaCl and 1 percent in Triton X-100 and then centrifuged at 270,000*g* through a cushion of the same solution containing 0.8*M* sucrose (lane 1). A similar protein profile was generated by collecting the material at the sucrose interface, diluting it with the above solution, adding NaCl to 2*M*, and centrifuging at 250,000*g* (b). Intermediate filaments were released from erythrocyte plasma membranes (from 15-day-old chicks) by a slight modification of a previously described method (*9*). Isolated membranes were washed with a solution of 10 m*M* tris-HCl, 2 m*M* EDTA, and 100 m*M* NaCl (*p*H 6.9 at 0°C) and then extracted with 1 m*M* ethanolamine at 0°C. Two-dimensional gels (*9, 29*) of a neurofilament preparation (b) and erythrocyte membrane sample (c) similar to those in (a), and their corresponding immunoautoradiograms (d and e, respectively) demonstrating the reactivity of the antiserum to NF70. This antiserum was elicited with NF70 cut from polyacrylamide gels (as in a, lane 1) (*11*). Coomassie blue was removed from the gel slices with dimethyl sulfoxide (DMSO) before immunization. Only portions of the original two-dimensional gels are shown in (b and c) corresponding to polypeptides with isoelectric points between 4.3 (left) and 6.2 (right) and larger than about 30K. The NF70 and E70 peptides often do not focus discretely, but either streak in the first dimension or cofocus with vimentin or the 68K heat shock protein (H). Maps of iodine-labeled chymotryptic peptides of NF70 (f) and E70 (g), showing identity. Mapping was performed according to Elder *et al.* (*14*), with the following modifications: Destained gel slices were freed of Coomassie blue with DMSO prior to iodination; iodination was quenched with 2 m*M* tyrosine, and gel slices were washed extensively with 1 m*M* tyrosine and 0.5*M* potassium iodide before digestion (the KI washing eliminates background spots that result from iodine labeling of the chymotrypsin by residual labeled iodide in the gel slice). Abbreviations: *NF205, NF170*, and *NF70* refer to the neurofilament "triplet" polypeptides; asterisks (*) are adjacent to the neuronal and erythroid subunits of spectrin; *H*, constitutive heat shock protein (*6*); *T*, α, and β tubulin; *G*, glial filament polypeptide; *A*, actin; *M*, a myelin polypeptide; *S*, synemin; *E70*, erythrocyte 70K polypeptide; and *V*, vimentin. Arrows point to corresponding positions of gels and autoradiograms.

primary structures of these two polypeptides.

Ultrastructural localization of E70 in chicken erythrocytes was accomplished be means of immunofluorescence and immunoelectron microscopy. Immunofluorescence microscopy reveals that E70 is a component of the intermediate filament network in these cells, with a fluorescence pattern similar to that seen with antibodies to vimentin and synemin (*9*). In most early definitive embryonic erythroid cells, as well as in many of the primitive series cells, individual intermediate filaments can be discerned easily with this antiserum (Fig. 2b). Later in embryogenesis, and for several weeks after hatching, most of the cells still react with this antiserum, but individual filaments usually cannot be traced; the fluorescence is confined to the region of the intermediate filament network (*10*), but usually appears diffuse rather than filamentous (Fig. 2d). In adults, most of the cells are negative for NF70, although a small percentage of cells exhibits diffuse fluorescence (Fig. 2f); individual filaments can be seen in only a small subset of these cells. The 4-week average lifespan of circulating erythrocytes (*15*) makes it unlikely that these positive cells are of embryonic origin. At all stages, there is a wide range of fluorescence intensities among the positive cells.

Immunoelectron microscopy confirms the filamentous localization of E70 in erythrocytes. The antibodies specifically decorate (bind to the surface of) the intermediate filaments (Fig. 2g). This decoration is not thick and uniform, as seen with antiserum to vimentin (*10*), nor regularly punctate, as with antiserum to synemin (*10*); rather, it appears irregularly punctate (Fig. 2g) when compared to the smooth profile of undecorated filaments (*10*). The amount of antibody bound per unit length of filament is variable among the different cells of a given population (compare Fig. 2, g and h), regardless of the age of the donor, although the amount of decoration appears relatively constant among filaments within a given cell; this is also evident by immunofluorescence (Fig. 2, b, d, and f). These observations suggest that the ratio of E70 and vimentin does not change appreciably within a given cell during the assembly of its intermediate filament network. The extent of decoration among cells of different ages correlates with the intensity of fluorescence as described above, being heaviest in embryonic cells and scant or absent in adult cells. The serum from unimmunized animals gives no decoration of the filaments. These labeling results are consistent with an observed age-related decline in the amount of E70, relative to vimentin, seen on polyacrylamide gels of erythrocyte membranes, intermediate filaments, and cytoskeletons; with a heavy loading of vimentin, E70 is prominent in samples from embryos and young chickens (Fig. 1) but is often undetectable in samples from adults.

Neither of the other two components of the neurofilament triplet (170K and 205K in our gel system) (*16*) has been detected in chicken erythrocytes. Neither polypeptide is apparent on gels of partially purified erythrocyte intermediate filaments (Fig. 1a); a high-titer antiserum against NF170, which has a slight cross-reactivity with NF205, does not detect antigen in various erythrocyte fractions as judged by immunoautoradiography, or binding by immunofluorescence or immunoelectron microscopy. Thus E70 exists in erythrocytes without these high molecular weight polypeptides with which it is usually associated in neurons. Similarly, no glial filament protein has been detected in erythroid cells with these same techniques (data not shown).

Axonal staining is the salient feature of frozen sections of various tissues when examined by immunofluorescence with the NF70 antiserum, but the erythrocytes throughout most of these sections are also clearly labeled when tissues from embryos and young chickens are examined (data not shown). However, the relative faintness of the erythrocytes compared to the axons, because of lower antigen density, may explain why NF70 has not been detected in erythrocytes previously.

The simplest interpretation of the antibody decoration data is that vimentin and E70 randomly copolymerize into intermediate filaments, and that modulation of the ratio of these two subunits in a given erythrocyte is a function of their differential synthesis. Evidence for copolymerization of different types of intermediate filament subunits has been accumulating for a variety of in vivo and in vitro systems (*17–19*), but it is not yet known how this assembly is regulated or if different subunits in heteropolymers exhibit any order. With regard to erythrocytes, the relative decline in the incorporation of E70 during development may be responsible for the reduction in the linear periodicity of the association of synemin with the core filament (*10*); addition of an E70 subunit to a vimentin polymer might lengthen the filament more than the addition of another vimen-

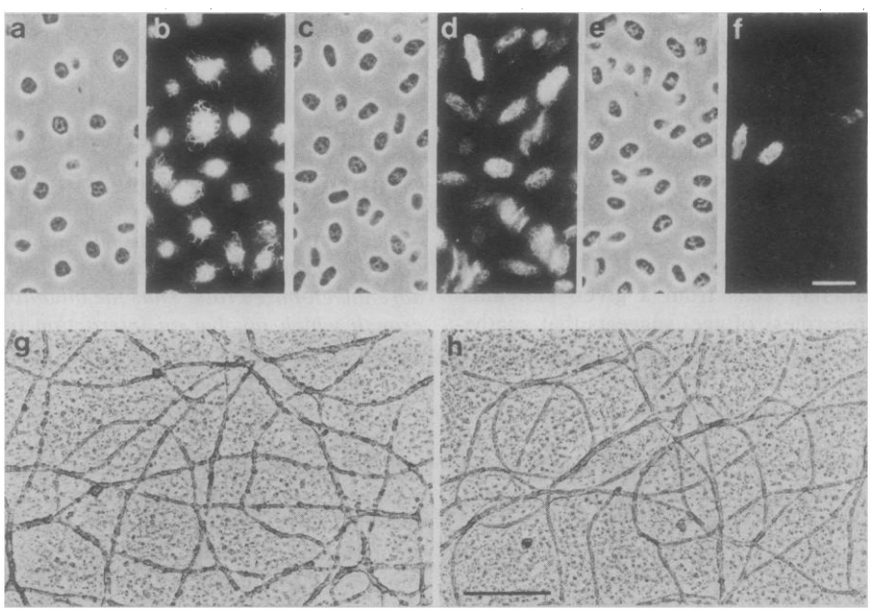

Fig. 2. Corresponding phase-contrast (a, c, and e) and fluorescence (b, d, and f) micrographs of cytoskeletons of circulating erythroid cells from an 8-day-old embryo (a and b), a 52-day-old chick (c and d), and an adult (13-month-old) chicken (e and f); cells were attached to cationized glass cover slips (*9*), lysed in a physiological buffer containing Triton X-100, and processed for indirect immunofluorescence using rabbit antiserum to NF70 and fluorescein-conjugated goat antiserum to rabbit immunoglobulin G (*9*). Only the nuclei are apparent in these phase-contrast micrographs. Electron micrographs (g and h) of platinum replicas of sonicated erythrocytes (*9, 10*) from a 19-day-old chick representing portions of cells that were near one another on the cover slip (and therefore processed identically); both were incubated with antiserum to NF70 before fixation, resulting in the specific decoration of the intermediate filaments with antibodies. The greater degree of decoration of the cell in (g) reflects a greater antigen density (a higher ratio of E70 to vimentin) in this cell. Bars are 10 μm for (a) to (f) and 500 nm for (g) and (h).

tin subunit, thereby increasing the distance between successive synemin binding sites.

The transient expression of the neurofilament polypeptide in erythrocytes is unlike the usual program of intermediate filament expression. Typically, in neurons, astrocytes, and muscle cells, vimentin is expressed initially but is largely or completely replaced during differentiation by NF70, glial filament protein, or desmin, respectively (*16, 17, 20*), except for some fetal ependymal cells in which glial filament protein is only transiently expressed (*21*). However, these cases are not strictly comparable to that of erythrocytes, since the neural, glial, and muscle changes involve the differentiation of individual cells rather than populations. Our results suggest that individual erythroid cells do not modulate their ratios of vimentin to E70 during differentiation, but that this ratio changes as the cell population changes during development and growth of the organism, culminating in the virtual absence of E70 from most cells in the adult. This is supported by the observations that phenylhydrazine-induced anemia in adult chickens does not result in circulating erythroblasts with increased amounts of E70, and that bone marrow from anemic and normal adult chickens contains little erythroid E70, as assayed by immunofluorescence (data not shown). Precedents exist for this time course of expression of avian erythroid components: A hemoglobin variant (*22, 23*) and certain cell surface antigens (*24, 25*) are present in embryos but disappear within a few months of hatching; furthermore, induced anemia does not result in their reappearance (*23, 25*). The observed range in the amount of E70 present in different cells from a given individual (regardless of age) suggests that synthesis of E70 may be influenced by hemopoietic microenvironments or may be a function of stem cell heritage.

The role of E70 in erythrocytes has not been discerned; it may modulate the structure or function of the vimentin-synemin filament network in these cells, or merely be an ontogenic vestige of gene expression. In the context of current concepts of intermediate filament expression, the existence of the neurofilament polypeptide in a nonneuronal cell type is surprising. Accumulating evidence that intermediate filament expression cannot always be correlated with the histological classification or embryonic derivation of cells indicates that, despite the general utility and validity of such a scheme, until the apparent exceptions are more fully understood, intermediate filament polypeptides may not always be suitable markers or indicators of cell type or state of differentiation.

Many unanticipated similarities between the nervous and hemopoietic systems have been described, such as an abundance of hemopoietic stem cells in the brain (*26*), common cell surface antigens, and responsiveness to neurohormones (*27*). Although the underlying reasons for these links remain elusive, the major neurofilament subunit can now be recognized as part of this phenomenon.

BRUCE L. GRANGER
ELIAS LAZARIDES
Division of Biology, California Institute of Technology, Pasadena 91125

References and Notes

1. E. Lazarides, *Nature (London)* **283**, 249 (1980); B. H. Anderton, *J. Musc. Res. Cell Mot.* **2**, 141 (1981); M. Osborn and K. Weber, *Cell* **31**, 303 (1982).
2. N. Geisler and K. Weber, *J. Mol. Biol.* **151**, 565 (1981); R. K. H. Leim and S. B. Hutchison, *Biochemistry* **21**, 3221 (1982); R. V. Zackroff, W. W. Idler, P. M. Steinert, R. D. Goldman, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 754 (1982).
3. M. Willard and C. Simon, *J. Cell Biol.* **89**, 198 (1981); G. A. Sharp, G. Shaw, K. Weber, *Exp. Cell Res.* **137**, 403 (1982).
4. P. N. Hoffman and R. J. Lasek, *J. Cell Biol.* **66**, 351 (1975); W. W. Schlaepfer and L. A. Freeman, *ibid.* **78**, 653 (1978).
5. C. Wang, D. J. Asai, E. Lazarides, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 1541 (1980).
6. C. Wang, R. H. Gomer, E. Lazarides, *ibid.* **78**, 3531 (1981).
7. M. Jacobs, Q. L. Choo, C. Thomas, *J. Neurochem.* **38**, 969 (1982).
8. I. Virtanen, M. Kurkinen, V.-P. Lehto, *Cell Biol. Int. Rep.* **3**, 157 (1979); C. L. F. Woodcock, *J. Cell Biol.* **85**, 881 (1980).
9. B. L. Granger, E. A. Repasky, E. Lazarides, *J. Cell Biol.* **92**, 299 (1982).
10. B. L. Granger and E. Lazarides, *Cell* **30**, 263 (1982).
11. ———, *ibid.* **22**, 727 (1980).
12. K. Burridge, *Methods Enzymol.* **50**, 54 (1978).
13. B. L. Granger and E. Lazarides, *Cell* **18**, 1053 (1979).
14. J. H. Elder, R. A. Pickett, J. Hampton, R. A. Lerner, *J. Biol. Chem.* **252**, 6510 (1977).
15. G. Hevesy and J. Ottesen, *Nature (London)* **156**, 534 (1945).
16. S. J. Tapscott, G. S. Bennett, Y. Toyama, F. Kleinbart, H. Holtzer, *Dev. Biol.* **86**, 40 (1981).
17. D. L. Gard and E. Lazarides, *Cell* **19**, 263 (1980).
18. R. A. Quinlan and W. W. Franke, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 3452 (1982); G. Sharp, M. Osborn, K. Weber, *Exp. Cell Res.* **141**, 385 (1982); E. D. Frank, G. P. Tuszynski, L. Warren, *ibid.* **139**, 235 (1982); W. Ip, S. I. Danto, D. A. Fischman, *J. Cell Biol.* **96**, 401 (1983).
19. P. M. Steinert, W. W. Idler, F. Cabral, M. M. Gottesman, R. D. Goldman, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 3692 (1981).
20. S.-H. Yen and K. L. Fields, *J. Cell Biol.* **88**, 115 (1981); K. Yokoyama, H. Mori, M. Kurokawa, *FEBS Lett.* **135**, 25 (1981); J. Schnitzer, W. W. Franke, M. Schachner, *J. Cell Biol.* **90**, 435 (1981); D. Dahl, D. C. Rueger, A. Bignami, K. Weber, M. Osborn, *Eur. J. Cell Biol.* **24**, 191 (1981).
21. U. Roessmann, M. E. Velasco, S. D. Sindely, P. Gambetti, *Brain Res.* **200**, 13 (1980).
22. T. A. Borgese and J. F. Bertles, *Science* **148**, 509 (1965); R. Fraser, B. Horton, D. Dupourque, A. Chernoff, *J. Cell. Physiol.* **80**, 79 (1972).
23. G. A. P. Bruns and V. M. Ingram, *Dev. Biol.* **30**, 455 (1973).
24. M. S. Osterhoudt and M. R. Irwin, *Vox Sang.* **10**, 493 (1965).
25. B. G. Sanders, *J. Exp. Zool.* **167**, 165 (1968); J.-P. Blanchet, *Exp. Cell Res.* **102**, 1 (1976).
26. P. F. Bartlett, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 2722 (1982).
27. E. S. Golub, *Nature (London)* **299**, 483 (1982).
28. U. K. Laemmli, *ibid.* **227**, 680 (1970).
29. P. H. O'Farrell, *J. Biol. Chem.* **250**, 4007 (1975).
30. Supported by grants from the NIH [T32 GM07616 (B.G.), RCDA (E.L.), BRSG RR07003, and GM06965], NSF, and MDA. B.G. was also supported by a postdoctoral fellowship from the MDA. We thank W. J. Nelson, J. Ngai, R. D. Owen, and J.-P. Revel for critical reading of the manuscript.

4 March 1983

Potent Interaction Between Glucocorticoids and Growth Hormone–Releasing Factor in vivo

Abstract. *Administration of dexamethasone significantly enhanced the pituitary growth hormone response to growth hormone–releasing factor in intact as well as adrenalectomized rats. Thus the inhibitory effects of glucocorticosteroids on somatic growth which involve an interaction of these steroids and growth hormone at a peripheral level may also involve a modification of pathways within the central nervous system that regulate normal growth hormone secretion.*

One of the most overt features of long-term treatment with glucocorticosteroids is the resultant inhibition of somatic growth in both man (*1*) and laboratory animals (*2*). Clinically, glucocorticoids suppress the pituitary growth hormone (GH) response to various stimuli (*3*). Yet these steroids increase the synthesis and content of GH in dispersed pituitary cells in vitro as well as sensitize the response of the somatotrophs to release GH after stimulation (*4*). As a result of the recent isolation and characterization of a growth hormone–releasing factor from a human pancreas tumor (hpGRF-44) which had caused acromegaly (*5*), we have been able to study this apparent dichotomy in the action of glucocorticoids on GH secretion. Rats subjected to adrenalectomy or sham operations were given long-term treatment with either saline or the synthetic glucocorticoid dexamethasone, and were then injected with two doses of hpGRF-44. Adrenalectomy, without steroid replacement therapy, significantly decreased the pituitary response to a submaximal dose of hpGRF-44, and the increase in plasma GH concentrations following the intravenous administration of hpGRF-44 was significantly greater in rats treated with dexamethasone. These results show that glucocorticosteroids enhance the GH response of the pituitary to hpGRF-44 in