cally active but which do not bind antibody 259 have been prepared recently; cells transformed by such mutants were not altered morphologically, nor did they show greatly inhibited thymidine incorporation, after injection of antibody 259. It is assumed that while viral oncogenes function without normal control, their mechanism of action is similar to that of related cellular genes. Here we have presented evidence to support this idea in the case of growth factor receptor-like molecules and related oncogenes. It is therefore possible that these data might aid in understanding the way in which cellular genes interact in the control of normal proliferation. Our results indicate that some receptor-like oncogenes depend on ras proteins to initiate some cytoplasmic oncogenes do not. There are, of course, numerous oncogenes which we have not yet tested which might behave differently from those described here. We propose that an important class of proliferative signals are received at the cell surface by receptor molecules such as growth factor receptors, and the c-ras proteins are essential in the transfer of these signals to cytoplasmic effectors having serine kinase activity; the effectors then modify target molecules which are directly involved in initiating a proliferative cycle. Accordingly, if the cytoplasmic effector were mutated such that it functioned without activation, proliferation would continue independently of c-ras proteins. Receptor molecules, on the other hand, would always require c-ras to stimulate proliferation.

While our data are consistent with the above scheme, they do not exclude many other possibilities involving multiple metabolic pathways and more complex interactions. For example, we have not reported results with nuclear oncogenes owing to their difficulty in transforming NIH 3T3 cells. The proposed scheme is primarily attractive because of its similarity to the carefully studied mechanism of signal transduction involving cyclic AMP. While it is unlikely that cyclic AMP itself regulates proliferation, G-regulatory proteins with enzymatic similarities to c-ras proteins are involved. These regulatory proteins control signal transduction from cell-surface receptors to cytoplasmic serine kinase effector molecules by regulating adenyl cyclase activity.

While the present study has examined only one aspect of what is likely to be a highly complex system for regulating proliferation, it does provide a means of functionally comparing separate viral oncogenes. Injection of antibody has been used in other studies to characterize the types of molecules responsible for tumour cell proliferation. Like NIH 3T3 cells transformed by mos or raf genes, many tumour cells show inhibition of proliferation when injected with anti-ras antibody. In this way their proliferation is distinct from that of the normal cell types studied, each of which was efficiently inhibited by the injected antibody.

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1. Bishop, J. M. Cell 42, 23–28 (1985).
2. Bishop, J. M. & Varums, H. E. in RNA Tumor Viruses: Molecular Biology of Tumor Viruses 2nd edn (eds Weiss, R., Teich, M., Varums, H. & Coffin, J.) 999–1108 (Cold Spring Harbor Laboratory, New York, 1984).
3. Doolittle, F. R. et al. Science 221, 275–277 (1983).
4. Downward, J. et al. Nature 307, 531–537 (1984).
5. Waterfield, M. D. et al. Nature 304, 35–39 (1983).
6. Shirer, C. J. et al. Cell 44, 665–676 (1985).
7. Ellis, R. W. et al. Nature 292, 506–511 (1981).
8. Swee, C. W. et al. Nature 312, 273–275 (1984).
9. McGrath, J. P., Capon, D. J., Gooddel, D. V. & Levinson, A. D. Nature 310, 644–649 (1984).
10. Klossner, W., Maxted, A. S. & Arlinghaus, R. B. Virology 138, 143–155 (1984).
11. Herskowitz, I., Heintz, B., Brent, M., Rapp, U. R. & Saltare, T. Nature 312, 558–561 (1984).
12. Parkhoff, J., Nigg, E. A. & Hunter, T. Cell 33, 161–172 (1983).
13. Kung, H.-F., Smith, M. R., Bekesi, E., Manne, V. & Stacey, D. W. Exp Cell Res 162, 363–371 (1986).
14. Mulshine, L. S., Smith, M. R. & Stacey, D. W. Nature 318, 241–243 (1985).
15. Furth, M. E., Davis, L. J., Fendellys, B. & Stoolman, L. M. J. Immunol 43, 294–304 (1982).
16. Stacey, D. W., DeGudichius, S. J. & Smith, M. R. (in preparation).
17. Brugger, I. S. & Eriksson, R. L. Nature 268, 346–348 (1977).
18. Fedele, L. A., Even, J., Garon, C. F., Donnast, L. & Stayer, C. J. Proc. natn. Acad. Sci. U.S.A. 78, 4036–4040 (1981).
19. Drummer, L., Fedele, L. A., Garon, C. F., Anderson, S. J. & Stayer, C. J. J. Immunol 41, 489–500 (1982).
20. Robbins, K. C., Derave, S. G., Reddy, P. E. & Aaronson, S. A. Science 218, 1113–1113 (1982).
21. Blair, D. G., McClements, W. L., Oxenase, M. K., Fishinger, P. J. & Vande Woude, G. F. Proc. natn. Acad. Sci. U.S.A. 77, 1504–1508 (1980).
22. Rapp, U. R. & Todaro, G. J. Proc. natn. Acad. Sci. U.S.A. 77, 624–628 (1980).
23. Hsu, J. R., Wood, T. G., Murphy, E. C., Blair, D. G. & Arlinghaus, R. B. Cell 28, 37–46 (1981).
24. Noda, M., Selinger, Z., Stoolman, E. M. & Bastin, R. H. Proc. natn. Acad. Sci. U.S.A. 80, 5602–5606 (1983).
25. Schiller, J. T., Vass, W. C. & Lowry, D. R. Proc. natn. Acad. Sci. U.S.A. 81, 7880–7884 (1984).
26. Papageorge, A. G. et al. Molec. cell. Biol. (in the press).
27. Beckner, S. K., Hattori, S. & Shit, T. Y. Nature 375, 71–72 (1985).
28. Gilman, A. Cell 36, 577–579 (1984).
29. Willger, M. et al. Proc. natn. Acad. Sci. U.S.A. 76, 1373–1376 (1979).
macrophages as well as a small proportion of the type I astrocyte.

Table 1  Induction of Ia antigen expression on glial cell cultures

| Treatment of primary glial cell cultures | Ia induction |
|----------------------------------------|-------------|
| Control (DMEM with 15% FCS)            | -           |
| Control conditioned media             | -           |
| Rat IFN-γ (10 U ml⁻¹)                  | +           |
| Rat IFN-γ + anti-rat IFN-γ (1,000 NU ml⁻¹) | -           |
| Infectious JHM virus (10³ PFU ml⁻¹)    | +           |
| Ultraviolet-inactivated JHM virus (10³ PFU ml⁻¹) | -           |
| JHM virus (glial or DBT cell-derived) + anti-rat IFN-γ | + |
| JHM virus + a non-neutralizing anti-IH antibody | + |
| JHM virus + a neutralizing anti-IH antibody | - |

+, Detectable by immunofluorescence microscopy (induction of Ia in at least 2,500-5,000 cells per cm²); —, undetectable by immunofluorescence microscopy (induction of Ia in 0-10 cells per cm²). DMEM, Dulbecco’s minimal essential medium; FCS, fetal calf serum. The stock preparation of recombinant rat IFN-γ contained 1.2 x 10⁶ U ml⁻¹ and 3 x 10³ U per mg protein. Polyclonal rabbit antisera to rat IFN-γ, given by Dr van der Meide, contained 1.0 x 10⁷ neutralizing units (NU) ml⁻¹. JHM virus was obtained from tissue culture supernatants of cells infected with wild-type JHM murine coronavirus. Virus supernatants were produced from two different sources, primary glial cultures and a cell line permissive for JHM (designated DBT). The amount of virus in the supernatants was determined by titration (as PFU ml⁻¹) on DBT cells. Stock virus from DBT cells numbered 2 x 10⁶ PFU ml⁻¹ and from primary glial cultures, 2 x 10⁵ PFU ml⁻¹, when the cytotoxic effect reached 90%. Virus preparations were completely inactivated with ultraviolet light (2,500 μW cm⁻²) for 5 min. Conditioned supernatants from infected cultures served as the control for the virus supernatant preparations. Monoclonal antibody directed against the envelope glycoprotein E2 has been described elsewhere. The neutralizing monoclonal antibody was used at a dilution sufficient to neutralize 10⁶ PFU ml⁻¹. Cultures were treated as indicated for 4 days, stained for Ia using OX6 monoclonal antibody, then examined by fluorescence microscopy as described in Fig. 2 legend. The total number of cells in 10-day cultures was, on average, 10⁶ cells cm⁻².

macrophages as well as a small proportion of the type I astrocyte population were induced to express Ia (Fig. 2d-f).

Treatment of primary glial cell cultures with either infectious or ultraviolet-inactivated JHM virus also induced Ia expression by astrocytes in a dose-dependent manner, peaking at 10⁶ plaque-forming units (PFU) ml⁻¹ (Table 1); higher concentrations had a toxic effect on the cells. JHM virus at 10⁷ PFU ml⁻¹ gave the maximum response regardless of the source (Table 1). Immunofluorescence microscopy of cultures treated with ultraviolet-inactivated JHM virus, using a polyclonal rabbit anti-serum to JHM virus, confirmed the absence of infected cells throughout the cultures, indicating that there was no JHM virus replication. Fluorescence-activated cell sorting showed that ~10% of all cells in the cultures became Ia-positive (Fig. 1b). Conditioned supernatants from infected cultures, diluted correspondingly, had no effect on Ia expression. In contrast, to rat IFN-γ, JHM virus induced Ia primarily in the astrocytic cell population (Fig. 2g-i), 90-100% of macrophages remaining negative, as determined by double immunofluorescence microscopy. In addition, the kinetics of induction was distinct from that seen with rat IFN-γ in that noticeable induction required at least 3-4 days of treatment, reaching a peak at 4-7 days. Double immunofluorescence analysis of GFAP and Ia showed that between 90 and 100% of the induced cells were astrocytes (Fig. 2g-i). In cultures treated with infectious JHM virus, the numerous Ia-positive astrocytes induced were apparently uninjected, as revealed by double immunofluorescence of Ia and JHM viral antigen. If indomethacin was added together with JHM virus, almost all the macrophages expressed Ia whereas macrophages without virus remained negative; this indicated that the ability of macrophages to express Ia was positively influenced by JHM virus, but that prostaglandins suppressed expression. Astrocytes appeared resistant to such suppression.

To determine whether the JHM virus had a direct effect on astrocytes or whether the effect was due to a secondary signal released by macrophages or astrocytes themselves, astrocytic cultures depleted of macrophages were tested for their responsiveness to JHM virus. Macrophages were removed by panning of trypanosized primary cultures on hydrophobic plastic; the relatively non-adherent astrocytes remaining in suspension were removed and re-plated. After 4 days of treatment with 10³ PFU ml⁻¹ ultraviolet-inactivated virus, these astrocytes expressed Ia, just as in cultures containing macrophages. Supernatants derived from either pure macrophage cultures or mixed primary cultures, after incubation with inactivated JHM virus, failed to induce Ia on naive astrocytes. This result indicated that secondary soluble factors were not involved in the induction of Ia antigen on astrocytes.

The possibility that Ia induction was the result of virus-induced interferon synthesis in the cultures was also examined. Primary cultures were treated with JHM virus (10⁷ PFU ml⁻¹, infectious or ultraviolet-inactivated) for 4 days, after which they
Fig. 2  a–c, Characterization of major cell types in one microscopic field by double immunofluorescence and phase-contrast microscopy of a 10-day control Lewis primary glial cell culture. a, Three Fc receptor+ (Fc+) macrophages with characteristic microspikes adhering to the substratum (arrows). b, One GFAP+ astrocyte (arrowhead) with fibrillar staining pattern characteristic of GFAP intermediate filaments. Cells were never double-labelled for Fc receptors and GFAP (see also ref. 15). c, Phase-contrast photomicrograph. Note the numerous lysosomal granules within the cytoplasm of Fc+ macrophages. Fc+ macrophages were found also to ingest large numbers of zymosan particles and to be nonspecific esterase-positive, both characteristic features of macrophages. d–f, Double immunofluorescence and phase-contrast microscopy of one microscopic field of a 10-day primary glial cell culture treated for 18 h with recombinant rat IFN-γ (10 U ml−1). d, Two macrophages (arrows) and one astrocyte (arrowhead) labelled for surface la. e, GFAP+ astrocytes with characteristic GFAP fibrillar staining pattern. Not all GFAP+ astrocytes are la-positive. The la+ macrophages in d are clearly negative for GFAP staining and can be identified by their characteristic microspikes (d) and lysosomal granules (arrows in f).  

f, Phase-contrast photomicrograph. The astrocyte indicated by an arrowhead in d–f is la+. g–i, Double immunofluorescence and phase-contrast microscopy of one microscopic field of a 10-day primary glial cell culture treated during the previous 4 days with 10^3 PFU ml−1 ultraviolet-inactivated JHM virus. g, Two astrocytes expressing cell-surface la (arrowheads). h, GFAP+ astrocytes showing a fibrillar staining pattern. The arrowheads in h and i pinpoint the two la+ cells that are indicated by arrowheads in g, showing strong double immunofluorescence of la and GFAP for the same cells. Note that the unlabelled GFAP+ astrocytes in h are not la-positive in g. i, Phase-contrast photomicrograph. The cell labelled with an arrow contains granules typical of macrophages and is negative for la (see g) and GFAP (h). The other granule-containing macrophage indicated by the crossed arrow shows only weak expression of la in g at the lower pole of its cell body bearing microspikes. Therefore, GFAP+ astrocytes are selectively induced to express la while over 90% of macrophages remain la−. Parallel cultures stained for JHM antigen revealed no infected cells throughout the cultures.

Methods. Primary glial cell cultures were established from newborn (1 day postnatal) Lewis rat brain as described previously36. Six days after plating, at which stage the cultures were treated with IFN-γ or JHM virus, three distinct cell populations were present: type I astrocytes, macrophages and A2B5+ precursors to both type II astrocytes and galactocerebroside-positive oligodendrocytes17. Staining of Fc receptors was achieved by incubating live cultures with a 1:100 dilution of normal mouse serum, followed by goat anti-mouse IgG conjugated to TRITC (Zymed, California), at 4°C (ref. 15). After fixation with 2% formaldehyde and permeabilization with 0.25% Triton X-100, GFAP filaments were stained using a polyclonal rabbit IgG directed against GFAP (Dakopatts, Denmark) diluted 1:250, followed by goat anti-rabbit IgG conjugated to FITC (Zymed). Staining of rat la and GFAP was as for Fc receptors and GFAP. A mouse monoclonal antibody directed against rat la (designated OX6; given by Dr D. W. Mason) was diluted to 20 μg ml−1 IgG from hybridoma supernatant. As a control for the OX6 monoclonal antibody which is of the IgG1 subclass, two different mouse monoclonal IgG1 antibodies against unrelated antigens were tested and found to be negative. Staining of JHM virus antigen was performed as for GFAP, using a rabbit IgG fraction directed against JHM, diluted to 20 μg ml−1 IgG.
The phenomenon reported here may represent a general feature of virus-astrocyte interactions and may have wider implications for human neurotropic viruses and the induction of Ia antigen on certain antigen-presenting cells. This unique expression pattern of Ia in a similar way to that described for bacterial endotoxin10.

The present results are particularly interesting as IFN-γ released by T lymphocytes is thought to be indispensable in the induction of Ia antigen on certain antigen-presenting cells1,12, including astrocytes. Astrocytes could be especially effective antigen-presenting cells in the brain owing to their ubiquity and their ability to phagocytose, process and present antigen1. In the case of virus invasion of the CNS, this cell population may play an important part in mounting an immune response to effectively control the viral infection. On the other hand, high constitutive levels of Ia expression might carry the risk of inappropriate presentation of self antigens, as is thought to occur in autoimmune processes directed against the thyroid gland. This phenomenon may have special relevance to brain antigens and Ia-expressing astrocytes as the development of immune tolerance to self brain antigens, including myelin, may be hampered by the blood-brain barrier. The induction of Ia on astrocytes by JHM virus probably has a role in the JHM virus-induced chronic demyelinating disease of Lewis rats, which involves induction of myelin basic protein-specific T lymphocytes. The phenomenon reported here may represent a general feature of virus-astrocyte interactions and may have wider implications for human neurotropic viruses and the induction of immunologically mediated chronic demyelinating diseases.

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1. Wong, G. H. W., Clark-Lewis, I., Harris, A. W. & Schrader, J. W. Eur. J. Immunol. 14, 52-56 (1984).
2. Hirsch, M. R., Wietzerbin, J., Pieters, M. & Gordin, C. Neurosci. Lett. 41, 199-204 (1983).
3. Fontana, A., Fierz, W. & Welterke, H. Nature 307, 237-238 (1984).
4. Wege, H., Siddel, S. & ter Meulen, V. Curr. Topics Microbiol. Immunol. 99, 165-189 (1982).
5. Wege, H., Wistuba, R. & ter Meulen, V. in Progress in Clinical & Biological Research. Experimental Allergic Encephalomyelitis: A Useful Model for Multiple Sclerosis (eds Abrols, E. C., Kies, M. W. & Skulling, A. J.) (Liss, New York, 1984).
6. Wistuba, R., Wege, H. & ter Meulen, V. Nature 308, 150-153 (1983).
7. Snyder, D. S., Boller, D. J. & Unanue, E. R. Nature 309, 165-167 (1982).
8. Walker, W. R., Maino, V., Sanchez-Lanier, M., Warner, N. & Stewart, C. J. Exp. Med. 159, 1532-1547 (1984).
9. Wege, H., Dörries, R. & Wege, H. J. Gen. Virol. 65, 1931-1942 (1984).
10. Monroe, J. G. & Cambier, J. C. J. Exp. Med. 158, 1589-1599 (1983).
11. Steeg, P. S., Moore, R. N., Johnson, H. M. & Oppenheim, J. J. J. Exp. Med. 156, 1780-1793 (1982).
12. Steeg, P. S., Johnson, M. & Oppenheim, J. J. J. Immunol. 129, 2402-2406 (1982).
13. Londei, M., Lamb, J. R., Rottazz, G. F. & Feldman, M. Nature 319, 639-641 (1984).
14. Traugott, U., Scheinbergh, L. C. & Ruiles, C. S. J. Neuroimmunol. 2, 1-14 (1982).
15. Raff, M. C., Fields, K. L., Hakomori, S. I. & Mirsky, R. Brain Res. 174, 283-308 (1979).
16. Mannu, P. T. & Moguillo, E. J. Neurochem. 14, 695-709 (1985).
17. Raff, M. C., Miller, R. H. & Noble, M. Nature 303, 390-396 (1983).
18. McMaster, W. R. & Williams, A. F. Eur. J. Immunol. 9, 426-433 (1979).

Major reorganization of immunoglobulin V_H segmental elements during vertebrate evolution

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In mammals, the immunoglobulin heavy-chain variable region (V_H) locus is organized in a linear fashion; individual V_H, diversity (D_H), joining (J_H) and constant (C_H) region segments are linked in separate regions. During somatic development, coding segments flanked by characteristic short recombination signal sequences, separated by intervening sequence regions that may exceed 2,000 kilobases (kb), are recombined. Combinatorial joining of different segments as well as imprecision in this process contribute to the diversity of the primary antibody response; subsequent mutation further alters functionally rearranged genes. This basic somatic reorganization mechanism is shared by six major families of genes encoding antigen receptors. Previously, we have shown that multiple germline genes and mammalian-like recombination signal sequences are associated with the V_H gene family of Heterodontus foscolensis (banded shark), a primitive elasmobranch. Here we demonstrate that segmental reorganization involving mammalian-like D_H and J_H segments occurs in the lymphoid tissues of this species. In marked contrast to the mammalian system, we find multiple instances of close linkage (~10 kb) between individual V_H, D_H, J_H and C_H segments. This unique organization may limit combinatorial joining and be a factor in the restricted antibody response of this lower vertebrate.© 1986 Nature Publishing Group