From the chemical shift data, it can be concluded that the compound that survives in the brain for at least 98 hours has retained the intact CF_3 group of the halothane molecule. Furthermore, there is no major change in the brain environment in which this molecule resides over this period. The long retention time suggests either the presence of a covalently bound halothane or conversion to a less volatile metabolite. Either possibility would be consistent with our preliminary mass spectrometric data. At this time we are unable to assign a structure to the long-lived species in the brain, although the ^{19}F NMR observation of a similar compound in blood, nerve, and muscle strongly suggests a nonvolatile species that can easily cross cell membranes.

In conclusion, we have directly observed the interaction of general anesthetics with brain tissue in a live mammal. We showed that by using ^{19}F NMR spectroscopy, one can detect small concentrations (100 to 500 μM) of fluorinated anesthetics in the brain during and after anesthesia. Spectra with good signal-to-noise ratios were obtained at 2-minute intervals, so it should be possible to follow the time course of anesthetic uptake and elimination as well as to detect the appearance of fluorine-containing metabolites in various organs in vivo over relatively short periods.

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2. Control experiments were performed in which the surface coil was placed directly on the surgically exposed frontal bones prepared under general anesthesia. The same results were obtained, confirming that the major source of the ^{19}F NMR signals was the brain. Examination of the scalp and other intervening tissues showed negligible amounts of ^{19}F signals.
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Identification of the c-*myc* Oncogene Product in Normal and Malignant B Cells

Abstract. *Antiserum to a synthetic peptide corresponding to the carboxyl-terminus of the human* c-myc *protein immunoprecipitated a 48,000-dalton protein from a number of normal and malignant human and mouse cells. The size of the protein is consistent with the potential coding region predicted from the* c-myc *nucleotide sequence, and is the same for malignant cells carrying either a rearranged or an unrearranged* c-myc *oncogene. Because* c-myc *transcripts are expressed at higher levels in malignant than in normal B cells, it appears that an increased level of the* c-myc *protein rather than a change in the gene product is the relevant factor in determining transformation.*

The human cellular homolog (c-*myc*) of the avian MC-29 viral transforming gene is normally located on band q24 of chromosome 8 (*1, 2*). In Burkitt lymphoma, the translocation involving chromosomes 8 and 14 [t(8;14)] (*3*) brings the c-*myc* gene in close proximity with the immunoglobulin heavy chain locus on chromosome 14 (*1*). We have previously found that the translocated c-*myc* gene in Burkitt lymphomas is either not rearranged, or rearranged head to head with the immunoglobulin constant region C_μ gene (*1, 4*).

The c-*myc* transcripts are expressed at higher levels in Burkitt lymphoma cells than in lymphoblastoid cells not carrying the translocation (*5, 6*). From further studies using somatic hybrids between mouse myeloma and Burkitt lymphoma cells carrying the chromosome with either the translocated or the untranslocated c-*myc* gene, and Burkitt cell lines carrying rearranged and unrearranged c-*myc* genes, we concluded that only the translocated gene is expressed in Burkitt lymphoma cells (*6, 7*). In all the cases examined, the length of the c-*myc* transcripts was approximately 2.3 kb (*5–7*).

In mouse plasmacytoma, the c-*myc* gene, which normally resides on chromosome 15, is translocated to the immunoglobulin heavy chain locus on chromosome 12 (*8, 9*). Mouse plasmacytomas in which the c-*myc* gene recombines with the C_α gene express novel 1.9- to 2.1-kb *myc* transcripts that are 0.4 kb shorter than the normal cellular *myc* transcripts (*8, 9*). The levels of c-*myc* transcripts are also elevated in mouse plasmacytoma (*8*).

The increased levels of c-*myc* transcripts in Burkitt lymphoma cells and mouse plasmacytomas suggest a possible role in transformation for the protein encoded by this oncogene. Therefore, we attempted to detect the c-*myc* product using antiserum to a synthetic peptide that corresponds to the carboxyl-terminus of the predicted protein (*10, 11*). Figure 1 shows the structure of the synthetic peptide and the corresponding protein sequence of human c-*myc* (*10, 11*). This peptide, prepared by the Merrifield solid-phase method (*12*), has 12 amino acids, with a tyrosine residue added at the amino end for coupling with the bovine serum albumin (BSA) carrier (*13*).

Rabbits were injected with the peptide-BSA conjugate, and given booster injections at 2-week intervals. Blood was withdrawn after the fourth booster injection. The antiserum thus obtained was assayed for the presence of antibodies to the *myc* gene product by immunoprecipitation of [^{35}S]methionine-labeled extracts of Burkitt lymphoma cells and human cell lines positive for c-*myc* transcripts by Northern blot or S1 nuclease analysis (*5–7*). The precipitates were analyzed by sodium dodecyl sulfate (SDS)–polyacrylamide gels (*14*), with serum from nonimmunized rabbits (preimmune serum) being used as a control. Immunoprecipitation inhibition experiments were carried out with antiserum that had been incubated with uncoupled peptide ($10^{-3}M$) to confirm that the amino acid sequence detected by the antibodies was the same as that of the peptide used for immunization.

The antiserum to the terminal peptide precipitated a 48,000-dalton polypeptide from Daudi, P3HR1, and JD38 IV lymphoma cells (Fig. 2A, lanes 2, 5, and 7,

respectively), which all carry the t(8;14) chromosome translocation, and from HL60 human promyelocytic leukemia cells (Fig. 2A, lane 10), which carry a normal but amplified c-*myc* oncogene (*15*). This protein was not precipitated by preimmune serum (Fig. 2A, lanes 1, 4, 6, and 9), and the precipitation was almost completely inhibited when the serum was incubated with the uncoupled peptide (Fig. 2A, lanes 3, 8, and 11). The 48,000-dalton protein was also detected in HeLa cells, and in the GM607 and GM2669 human lymphoblastoid cell lines, which carry a normal c-*myc* gene, and in Colo 320 human colon carcinoma cells (Fig. 2B, lanes 2, 4, 6, and 8), which carry an amplified c-*myc* gene (*16*). All of these cell lines express c-*myc* RNA (*5–7, 11, 15*). Immunoprecipitation of the 62,000-dalton polypeptide that appeared nonspecifically was not inhibited by the synthetic peptide (data not shown).

The antiserum also precipitated a 48,000-dalton protein from the [^{35}S]cysteine-labeled lysate of mouse myeloma cells and from a hybrid cell line (mouse myeloma × Burkitt lymphoma cells) that carries the translocated human c-*myc* oncogene (Fig. 3A, lanes 2 and 4, respec-

A

NH_2.....Gln-Leu-Lys-His-Lys-Leu-Glu-Gln-Leu-Arg-Asn-Ser-Cys-Ala

B

NH_2-Tyr*-Lys-His-Lys-Leu-Glu-Gln-Leu-Arg-Asn-Ser-Cys-Ala

Fig. 1 (left). Carboxyl-terminal sequence of (A) the c-*myc* gene product and (B) the synthetic peptide used for immunization. The peptide was produced with a Vega 250 peptide synthesizer. The crude product was purified by gel filtration and reverse-phase chromatography. The major fraction obtained was judged to be pure by amino acid composition and sequence. The tyrosine residue was added for coupling purposes. Fig. 2 (right). Immunoprecipitation of human c-*myc*-encoded protein and inhibition with synthetic peptide. (A) [^{35}S]Methionine-labeled lysates from Burkitt lymphoma cells, Daudi (lanes 1, 2, and 3), P3HR-1 (lanes 4 and 5), JD38 IV (lanes 6, 7, and 8), and HL60 promyelocytic leukemia cells (lanes 9, 10, and 11). Lanes 1, 4, 6, and 9: preimmune serum. Lanes 2, 5, 7, and 10: antiserum to the synthetic peptide. Lanes 3, 8, and 11: immune serum after adsorption with carboxyl-terminal peptide. (B) [^{35}S]Methionine-labeled lysates from HeLa cells (lanes 1 and 2), GM607 (lanes 3 and 4) and GM2669 (lanes 5 and 6) human lymphoblastoid cells, and Colo 320 colon carcinoma cells (lanes 7 and 8). Lanes 1, 3, 5, and 7: preimmune serum. Lanes 2, 4, 6, and 8: antiserum to the synthetic peptide.

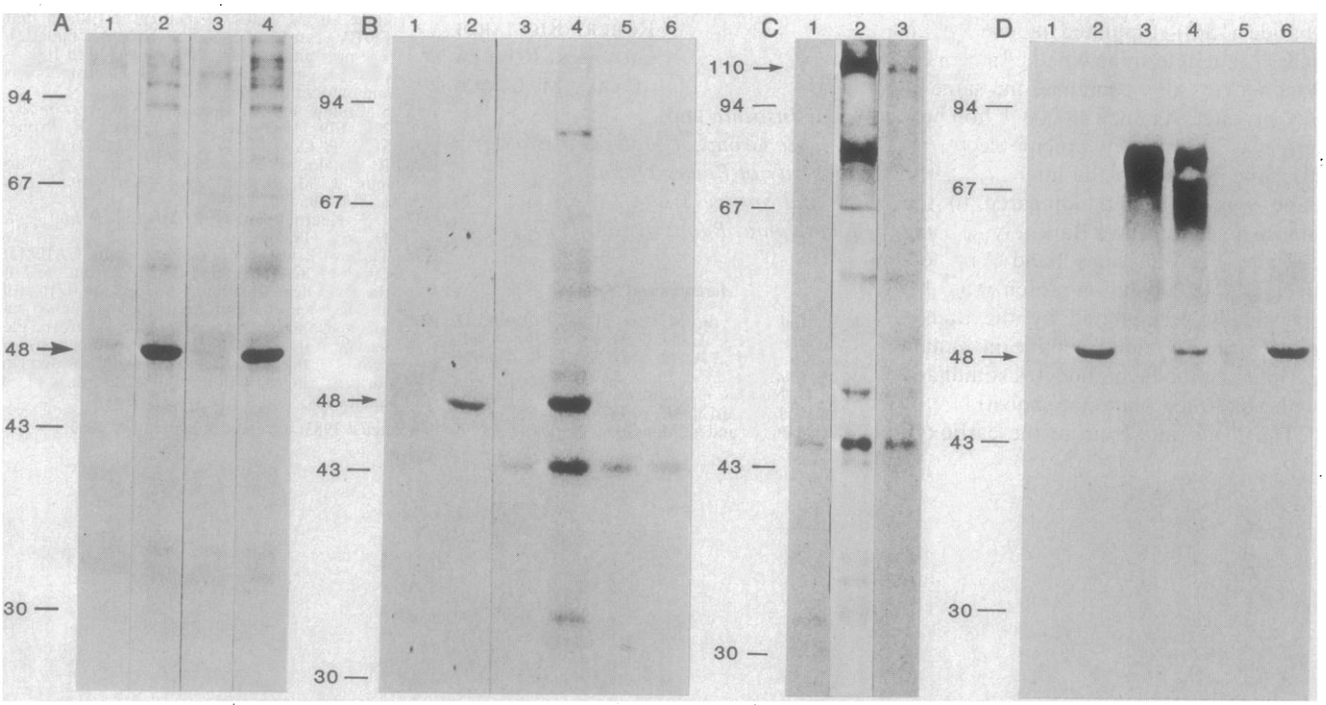

Fig. 3. (A to C) Immunoprecipitation of c-*myc* protein from mouse, human, and hybrid cell lines and the gag-*myc* fusion protein from MC29-transformed quail cells. (A) [^{35}S]Cysteine-labeled lysates from mouse myeloma cells NP3 (lanes 1 and 2), and mouse myeloma × Burkitt lymphoma hybrid cells JEID6 (lanes 3 and 4). Lanes 1 and 3: preimmune serum. Lanes 2 and 4: antiserum to the synthetic peptide. (B) [^{35}S]Methionine-labeled lysates from mouse myeloma cells NP3 (lanes 1 and 2), Burkitt lymphoma cells CA46 (lanes 3 and 4), and human breast carcinoma cells SKBR 5 (lanes 5 and 6). Lanes 1, 3, and 5: preimmune serum. Lanes 2, 4 and 6: antiserum to the synthetic peptide. (C) [^{35}S]Methionine-labeled lysates from the nonproducer line of MC29-transformed quail embryo fibroblasts, Q8. Lane 1: preimmune serum. Lanes 2 and 3: antisera to p19 and the synthetic peptide, respectively. (D) Immunoprecipitation of c-*myc* protein from lipopolysaccharide (LPS)-stimulated mouse spleen cells and comparison with mouse myeloma and Burkitt lymphoma cell lines. Spleen cells from 4- to 6-week-old BALB/c mice were cultured at 5×10^5 cells per milliliter with LPS (*Salmonella typhosa*) (50 μg/ml) in RPMI 1640 medium supplemented with 20 percent fetal calf serum for 4 days. [^{35}S]Cysteine-labeled lysates from NP3 mouse myeloma cells (lanes 1 and 2), LPS-stimulated mouse spleen cells (lanes 3 and 4), and CA46 Burkitt lymphoma cells (lanes 5 and 6). Lanes 1, 3, and 5: preimmune serum. Lanes 2, 4, and 6: antiserum to the synthetic peptide. The same amount of radioactivity (counts per minute) was used for each immunoprecipitate.

tively) and expresses very high levels of mouse and human c-*myc* transcripts (*6*). Thus, the c-*myc* gene encodes a protein of the same size in mouse and human cells. As expected, the antiserum did not precipitate any specific protein from a human breast carcinoma cell line SKBR 5 (Fig. 3B, lane 6) in which no c-*myc* transcripts were detectable by Northern blotting analysis (*11*). In the same experiment, mouse myeloma cells and a Burkitt lymphoma cell line served as positive controls (Fig. 3B, lanes 2 and 4, respectively). We also precipitated the lysate of MC29-transformed Q8 quail cells with either antiserum to the p19 protein of avian leukosis virus or with the antiserum to the *myc* peptide. As shown in Fig. 3C (lane 2), the antiserum to p19 precipitated the fusion protein between the group specific antigen of avian leukosis virus and the *myc* gene product (p110). The antiserum to the *myc* peptide also precipitated the p110 protein (Fig. 3C, lane 3), but the low intensity of this p110 band is probably due to the low affinity of the antibodies for the viral protein, since there is a difference in two amino acids between the last 12 amino acids of the human and avian viral *myc* gene products. We then tested the antiserum on lipopolysaccharide (LPS)-stimulated mouse spleen cells (*17*) to determine whether normal B lymphocytes also contained the same c-*myc* product. Again, a 48,000-dalton protein was specifically precipitated (Fig. 3D, lane 4), though the intensity of the band was decreased compared to the mouse myeloma and a Burkitt lymphoma cell line (Fig. 3D, lanes 2 and 6, respectively). A 48,000-dalton protein was also specifically precipitated by the antiserum to the synthetic peptide in human peripheral blood lymphocytes stimulated with pokeweed (data not shown).

Thus, our antiserum to the carboxyl-terminus of the predicted c-*myc* protein enabled us to identify the c-*myc* gene product as a 48,000-dalton protein. The size of the protein specifically immunoprecipitated is consistent with the nucleotide sequence of the human c-*myc* gene that suggests a messenger RNA with a coding capacity for a 48,812-dalton protein (*10, 11*). We detect a c-*myc* protein of the same size in normal and malignant B cell lines, independent of c-*myc* gene rearrangement.

Thus, the absence of any detectable size difference in the c-*myc* gene product in normal and malignant cells, together with the high levels of c-*myc* transcripts observed in Burkitt lymphomas (*5, 6*), strongly suggest that an elevated constitutive expression of this protein is responsible for the expression of malignancy. Studies to quantitate the levels of expression of the *myc* gene product in normal and malignant B cells will resolve this issue.

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14. Cells were labeled with [^{35}S]methionine or [^{35}S]cysteine (100μCi/ml). After incubation for 30 to 90 minutes, the cells were washed with phosphate-buffered saline and lysed in 1 ml of extraction buffer (50 m*M* tris-HCl, *p*H 8, 0.15*M* NaCl, 5m*M* EDTA, 0.5 percent NP40, 2 m*M* phenylmethylsulfonide fluoride). The extract was briefly sonicated and centrifuged for 1 hour at 35,000 rev/min. Portions (300 μl) of cell lysate were adsorbed on ice with fixed *Staphylococcus aureus*. The cleared lysate was incubated with 10 μl of antiserum for 1 hour at 4°C. Immunoprecipitates were adsorbed on fixed *Staphylococcus aureus* and washed five times with washing buffer (15 m*M* tris-HCl, *p*H 7.4, 5 percent sucrose, 0.5*M* NaCl, 1 percent NP40, 5 m*M* EDTA, 2 m*M* phenylmethylsulfonyl fluoride). The precipitates were then subjected to 10 percent SDS-polyacrylamide gel electrophoresis and autoradiography.
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