ant mutants. It may be that mutant analysis will show that there is more than one mechanism of anesthetic action in *C. elegans*.

These experiments will help define the genetic control of anesthetic response but will not in themselves determine the mechanism of anesthetic action. To do that, we hope to use the variety of genetic and molecular tools available with *C. elegans*, such as making genetic mosaics (*15*) and cloning the genes (*16*).

REFERENCES AND NOTES

1. I. Ueda, *Anesth. Analg.* **63**, 929 (1984).
2. J. E. Sulston, E. Schierenberg, J. G. White, J. N. Thomson, *Dev. Biol.* **100**, 64 (1983); J. E. Sulston and H. R. Horvitz, *ibid.* **56**, 110 (1977).
3. J. G. White, E. Southgate, J. N. Thomson, S. Brenner, *Phil. Trans. R. Soc. London Ser. B* **275**, 327 (1976); J. G. White, *Trends Neurosci.* **8**, 277 (1985).
4. E. M. Hedgecock, J. G. Culotti, J. N. Thomson, L. A. Perkins, *Dev. Biol.* **111**, 158 (1985); J. G. White *et al.*, *Nature (London)* **297**, 584 (1982).
5. P. G. Morgan and H. F. Cascorbi, *Anesthesiology* **62**, 738 (1985).
6. H. R. Horvitz, S. Brenner, J. Hodgkin, R. K. Herman, *Mol. Gen. Genet.* **175**, 129 (1979).
7. We mapped *ec1* by mating *unc-79 (ec1)* males to a set of autosomal *dpy* mutations, *dpy-5 (e61) I, dpy-10 (e128) II, dpy-18 (e364) III, dpy-13 (e184) IV*, and *dpy-11 (e224) V*, then picking the non-Dpy F_1 progeny and allowing them to self-fertilize. From these, the presence of halothane-hypersensitive Dpy F_2 was determined; such *dpy unc-79* animals were rare only for *dpy-18*, which implied that *ec1* is on LG III. Because *ec1* resembled *unc-79 (e1068)* in gross movement, a complementation test was done by mating *unc-79 (e1068)* hermaphrodites with *ec1*/+ males and showing that half the male progeny (59 of 130) were both uncoordinated and hypersensitive to halothane. A double mutant strain was constructed with another *dpy* marker on linkage group III, *dpy-17 (e164)*, and a two-factor map distance was determined. From this, *ec1* was found closely linked to *dpy-17*, a result consistent with the known map position of *unc-79* (*8*).
8. M. M. Swanson, M. L. Edgley, D. L. Riddle, *Genet. Maps* **3**, 286 (1984).
9. J. A. Lewis, personal communication.
10. We mapped *unc-80* by a strategy similar to that for *ec1*, described above (*7*). From the initial tests, *unc-80* was found to be linked, although loosely, to *dpy-11* on linkage group V. It was mapped further using *dpy-21 (e428)* for two-factor mapping and by mating *dpy-21 unc-80* males to *unc-51 (e369)* for three-factor mapping; *unc-80* maps between *dpy-21* and *unc-51*.
11. A population of worms was soaked in a solution containing 25 μl of EMS in 5 ml of M9 buffer (*5*) for about 4 hours, with occasional agitation; final EMS concentration was 25 m*M*. The worms were then picked individually onto plates where they were allowed to self-fertilize for two generations before scoring.
12. We mapped *ec27* to the X chromosome by showing that mutant F_1 males resulted when wild-type males were mated to *ec27* hermaphrodites. The location of *ec27* on the X was determined by using the duplications *mnDp8, mnDp10*, and *mnDp25* (*8*). A complementation test was done by mating *mnDp25/+; unc-9 (e101)/0* males to *unc-9(ec27) dpy-6* hermaphrodites. The resulting non-Dpy hermaphrodites were uncoordinated.
13. J. Hodgkin, *Genetics* **103**, 43 (1984).
14. M. Sedensky, P. Morgan, P. Meneely, unpublished results.
15. R. K. Herman, *Genetics* **108**, 165 (1984).
16. I. Greenwald, *Cell* **43**, 583 (1985).

17. This work is a continuation of the work of P. Morgan, whom we thank for strains, advice, and discussion. We also thank J. Lewis for telling us about *unc-80* and for helpful discussions, K. Nordstrom for technical assistance, and S. Henikoff, P. Morgan, and H. Cascorbi for comments on the manuscript. We thank R. Haschke and members of his laboratory for the use of the gas chromatograph. Some of the strains used in this work were provided by the *Caenorhabditis* Genetics Center, which is supported by a contract between the National Institute on Aging and the University of Missouri. We thank M. Edgley of the *Caenorhabditis* Genetics Center. Supported by NSF grant DCB-8417215 and March of Dimes grant 5-525 (to P.M.) and by NIH training grant GM-07604 (to M.S.).

10 November 1986; 24 March 1987

Tissue-Specific Expression of Functionally Rearranged λ1 Ig Gene Through a Retrovirus Vector

ROGER D. CONE,* EDWARD B. REILLY, HERMAN N. EISEN, RICHARD C. MULLIGAN

To explore the potential use of retrovirus vectors for the transfer of genomic DNA sequences into mammalian cells, recombinant retroviral genomes were constructed that encode a functionally rearranged murine λ1 immunoglobulin gene. Several of these genomes could be transmitted intact to recipient cells by viral infection, although successful transmission depended both on the orientation of the λ1 sequences and on their specific placement within vector sequences. The λ1 gene transduced by viral infection was expressed in a cell lineage–specific manner, albeit at lower levels than endogenous λ1 gene expression in cells from the B-lymphocyte lineage. Vectors yielding integrated proviruses that lacked viral transcriptional enhancer sequences were used to show that neither viral transcription nor the viral transcriptional sequences themselves had any effect on the tissue specificity of λ1 gene expression or the absolute amount of λ1 transcription. Vector transcription did, however, dramatically decrease the amount of λ1 protein that could be detected in transduced cells. These results suggest that retrovirus vectors may be useful reagents not only for the expression of complementary DNA sequences but also for studies of tissue-specific transcription in mammalian cells.

STUDIES OF THE TISSUE-SPECIFIC expression of cloned genes depend on both the means for introducing the desired sequences into appropriate cell types and the placement of those sequences in a chromosomal (or intranuclear) context compatible with the mechanisms that normally regulate gene expression. Although much success has been achieved in the development of methods for introducing genes into different kinds of mammalian cells, little is known about whether particular methods of gene transfer per se can influence the expression of inserted sequences. Since retrovirus vectors represent a versatile gene transfer system for introducing sequences into a wide range of recipient cells, we asked in this study whether retroviral-mediated gene transfer could be used to transfer genomic DNA sequences to cells and whether expression of such sequences within the context of an integrated provirus might differ in a qualitative fashion from the expression of sequences transferred by conventional DNA transfection techniques. A functionally rearranged gene encoding a murine λ1 immunoglobulin light chain was examined in the study presented here. Although endogenous λ1 genes are expressed at extremely high levels in plasma cells or myeloma cell lines, we and others have been unable to obtain the regulated expression of cloned λ1 genes after their transfer to cells by conventional gene transfer methods (*1*). In addition, no evidence for enhancer-like elements in the intron between the Jλ and Cλ gene segments has been found in contrast to those found in the κ and heavy-chain immunoglobulin genes (*1*).

Because previous reports have described difficulties in constructing retrovirus vectors with complete transcriptional units encoded within a provirus (*2*), we initially tested four constructs in which the λ1 sequences were introduced in both orientations into each of two sites in the vector pZIP-NeoSV(X)1 (*3*) (Fig. 1). In each of these constructs the insert was a 4.4-kb rearranged λ1 gene, containing the leader, joined V-J gene segments, and C gene segment with the normal two λ1 introns as well as approximately 250 bp of 5′ untranslated nucleotides and 2000

R. D. Cone and R. C. Mulligan, Whitehead Institute for Biomedical Research, Cambridge, MA 02142, and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.
E. B. Reilly and H. N. Eisen, Center for Cancer Research, Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.

*Present address: Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724.

bp of 3′ untranslated nucleotides. The constructs were transfected into the helper-free virus–packaging cell line, ψ2 (*4*), and several G418-resistant clones were isolated from each transfection. The clones were all tested for virus production, as described previously (*3*). Only the construct (pSVXλ1 8), in which the λ1 gene was inserted at the Bam HI site between the viral splice donor and acceptor, and in reverse transcriptional orientation to the provirus, was capable of consistently producing full-length virus. Southern hybridization analysis of DNA from infected, G418-selected NIH 3T3 cells demonstrated that infection with virus from pSVXλ1 8 yielded full-length proviruses by comparison with the parent plasmid. Occasional ψ2 clones that produced virus, which resulted from transfection with the other three constructs (pSVXλ1 5, pSVXλ1 7, or pSVXλ1 10), expressed virus that had invariably deleted all or part of the λ1 gene.

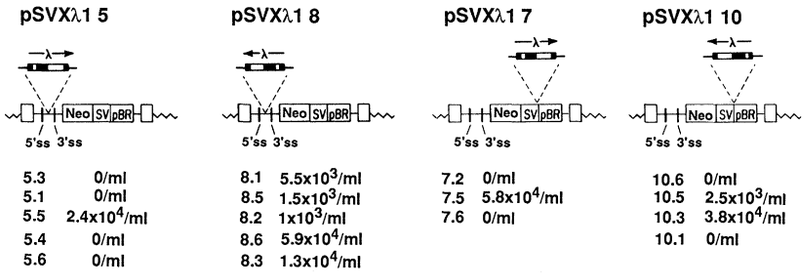

Fig. 1. Viral titers produced by ψ2 clones transfected with retroviral vectors containing inserts of a functionally rearranged murine λ1 immunoglobulin gene. The 7.4-kb Eco RI fragment encoding the rearranged λ1 gene from HOPC2020 (*7*) was partially digested with Xba I, and the 4.4-kb gene fragment was expanded by ligating with Eco RI linkers and subcloning into pBR322. To construct the four pSVXλ1 vectors, the 4.4-kb Eco RI fragment was inserted in both orientations at the Eco RI site, between the SV40 (SV) and pBR322 (pBR) replication origins, or ligated with Bcl I linkers and inserted in both orientations at the Bam HI site, located between the viral splice donor [5′single-stranded (ss)] and acceptor sites (3′ss). These four vectors were transfected into the helper virus–free packaging line, ψ2 (*4*), and several G418-resistant ψ2 clones from each transfection were expanded. Viral supernatants were prepared from each clone and titered for the ability to confer G418 resistance on NIH 3T3 cells. Titers are given in G418-resistant colony-forming units per milliliter. The pSVXλ1 8 vector was used for subsequent experiments. The darkened areas in the λ1 insert correspond to L, V-J, and C encoding regions, respectively, whereas the clear regions represent the two introns.

The pSVXλ1 8 construct contained the λ1 gene inserted between the viral splice donor and acceptor sequences. In wild-type Moloney virus–infected cells, approximately one-half of the viral RNA remains unspliced, and pZIP-NeoSV(X)1 was constructed under the assumption that this regulated splice could be used to express both full-length viral RNA for transmission of the viral genome and spliced RNA, in which sequences inserted at the Bam HI site were removed. In the spliced RNA the positioning of the *gag-pol* messenger RNA (mRNA) leader sequence next to the neomycin phosphotransferase gene would encode G418 resistance.

We tested this hypothesis by Northern blot analysis of RNA from a number of cell lines infected with virus from ψ2 clones that produced pSVXλ1 8 (Fig. 2). Five different cell types expressed the full-length and spliced messages at approximately equal levels, as was seen by hybridization of the filter with a ^{32}P-labeled *neo* gene probe. The spliced message should not have contained any λ1 gene sequences and, when the filter was rehybridized with a λ1 gene probe, only the full-length transcript was detected.

We next assayed the same infected cell populations for λ1 light-chain message (Fig. 3). Equivalent amounts of total cellular RNA from B18, a λ1-producing hybridoma (lane a), pSVX λ1-infected NIH 3T3 cells, (lanes b and c), infected EL4 cells (T lymphoma cell line) (lane d), infected 1881Y and 70Z cells (pre–B cells) (lanes e and f), and infected X63.Ag8.653 cells (myeloma cells) (lane g) were analyzed by Northern hybridization with a λ1 gene probe. Although nearly equivalent levels of viral RNA were seen in all the infected cells (3-hour exposure), we detected no λ1 mRNA in NIH 3T3 cells, a little in the T-cell line, and significantly more in the three lines of B-cell lineage: 1881Y, 70Z, and X63.Ag8.653 (inset, 72-hour exposure). The λ1 mRNA produced by the infected cells of B-cell lineage coelectrophoresed with authentic λ1 mRNA from the B18 hybridoma (lane h), although the absolute levels of mRNA expressed by the transduced gene were 1/100 as much as the levels from the hybridoma.

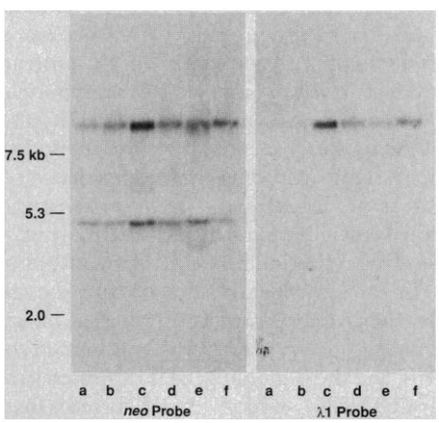

Fig. 2. Expression and regulated splicing of full-length viral RNA in cells infected with the pSVXλ1 8 vector. NIH 3T3 cells (*8*), 1881Y Abelson virus–transformed pre–B cells (*9*), 70Z B-lymphoma cells (*10*), EL4 T-lymphoma cells (*11*), and the X63.Ag8.653 myeloma line (*12*) were infected with virus from ψ2 pSVXλ1 8.3 or ψ2 pSVXλ1 8.6, and selected by growth in G418-containing medium. Total cellular RNA was prepared (*13*) from each infected population, and equal amounts (20 μg) were electrophoresed on a 2.2*M* formaldehyde 1% agarose gel. The gel was transferred to a Zetabind filter (AMF/cuno), which was hybridized with a ^{32}P-labeled *neo* gene probe (**left panel**), washed to remove all radioactive label, and then rehybridized with a ^{32}P-labeled λ1 gene (LVJC) probe (**right panel**). Markers, visualized by ethidium bromide staining, are 18*S* ribosomal RNA (rRNA) (2.0 kb), 28*S* rRNA (5.3 kb), and polio RNA (7.5 kb). Full-length viral transcripts are at 8.7 kb, and the spliced transcript is at 4.1 kb. Lanes a, NIH 3T3 cells with 8.3; lanes b, NIH 3T3 cells with 8.6; lanes c, 1881Y cells with 8.6; lanes d, 70Z cells with 8.6; lanes e, EL4 cells with 8.6; and lanes f, and X63.Ag8.653 cells with 8.6.

Although significant levels of λ1 mRNA were detected in cells of B lineage after infection with pSVXλ1 8 (0.05 to 0.1% of the polyadenylated [poly(A)$^+$] RNA), attempts to detect λ1 light-chain protein from these cells by metabolic labeling and immunoadsorption with a monoclonal antibody to λ1 chains were all negative (for example, Fig. 4, lane b). However, we had noted that the ratio of viral transcripts to λ1 transcripts was roughly 15:1, and this raised the possibility that the excess "antisense" viral RNA, with its included antisense λ1 RNA, hybridized to and blocked the translation of the λ1 message (*5*). To test this hypothesis, we generated a construct identical to pSVXλ1 8 except for a deletion removing the viral enhancer sequences from the 3′ long terminal repeat (LTR). This vector, pSVXλ1en$^-$, was transfected into ψ2 cells, and G418-resistant producer clones were expanded. Transfected pSVXλ1en$^-$ DNA is transcriptionally active and is able to encode G418 resistance because the 5′ LTR still retains enhancer sequences. However, since the enhancer lies 5′ to the promoter in each LTR, transcription of pSVXλ1en$^-$ yields a viral genome that lacks all enhancer sequences, and reverse transcription of this genome produces a provirus that is transcriptionally inactive as a consequence of being enhancerless.

As expected, infection of murine NIH 3T3 cells with pSVXλ1en$^-$ virus yielded more than ten G418-resistant colonies of viral supernatant per milliliter. However,

infection of the myeloma cell line X63.Ag8.653 yielded over 10^4 G418-resistant colonies per milliliter. Northern blot analysis of infected myeloma cells with the pSVXλ1en$^-$ virus indicated that the absolute amount of λ1 RNA produced was comparable to that found in pSVXλ1 8–infected cells. In contrast, the amount of viral RNA, although detectable, was approximately 1/15 the amount found in pSVXλ1 8–infected cells. Therefore, approximately equal amounts of viral RNA and λ1 RNA were found in pSVXλ1en$^-$ cells, in contrast to the 15:1 ratio of viral to λ1 RNA found in cells infected with the en$^+$ construct. In contrast to results obtained with the en$^+$ construct, metabolic labeling of pSVXλ1en$^-$-infected X63.Ag8.653 cells with [^{32}S]methionine followed by immunoadsorption yielded readily detectable levels of λ1 light-chain protein (Fig. 4, lane e). This result indicates that the level of viral RNA influences the amount of λ1 protein produced. Further, the results may suggest that the inserted λ1 sequences can activate the enhancerless proviral transcriptional unit in a tissue-specific fashion.

Overall, the work presented here indicates that retrovirus-mediated gene transfer may complement existing methods for studying transcriptional regulation in mammalian cells. Although there do appear to be constraints on the construction of recombinant retroviral genomes containing genomic sequences, there does not appear to be any inherent incompatibility between inserted transcriptionally active sequences and proviral transcription. The need to orient genomic sequences in a direction opposite to that of the proviral transcriptional unit is expected, on the basis of the demonstrated ability of functional polyadenylation sites to prematurely terminate genomic-length viral transcription. The effect of the insert's placement within vector sequences is less well explained but may depend on configurations that interfere with the generation of the subgenomic vector transcript essential for G418 selection.

Although the presence of active viral transcriptional signals does not appear to affect the transcription of λ sequences in either a quantitative or qualitative way, the signals do appear to affect λ1 protein production. Even though these results are reminiscent of a number of recent antisense experiments (*5*), they differ from those experiments in several potentially important ways. First, the ratio of antisense to "sense" RNA necessary to produce a major effect on λ1 protein products is low, compared to that reported by others (*5*). Second, and more important, in the studies presented here, the transcriptional units encoding the complementary RNAs are overlapping. Such a transcriptional configuration may facilitate more efficient hybridization between the two RNAs.

In another similar study, we have shown that retroviral constructs carrying an intact human β-globin gene can be transmitted to cells, and the β-globin gene is expressed in a tissue-specific fashion (*6*). Whereas that study also suggests the utility of retrovirus vectors for investigating transcriptional regulation in mammalian cells, the results presented here provide considerably more insight into specific features of the constructs that account for their successful use, and the data suggest experimental contexts in which the use of retroviral vectors may be particularly important. In the present study, for example, it was possible to document the effect of viral transcription on the insert's expression at the protein level, while in the case of the globin experiments, the excess amount of endogenous mouse β-globin protein precluded such an analysis. In addition, the results obtained with the enhancer-deficient λ1 vectors in myeloma cells suggest that the vectors may be useful for assaying and identifying regulatory sequences with an inserted gene. In the case of the β-globin experiments, this was not possible, since the viral transcriptional unit itself and the inserted β-globin sequences responded similarly in erythroid cells. Finally, and perhaps most importantly, the present study illustrates that the mode of gene transfer can influence the expression of a cloned gene after its introduction into mammalian cells. This may suggest that chromosomal sites into which retroviruses integrate are fundamentally different from sites into which DNA segments integrate after DNA transfection, or that some of the structural features of the inserted sequences themselves (for example, chromatin structure) are dictated by the mode of gene transfer and can influence gene expression.

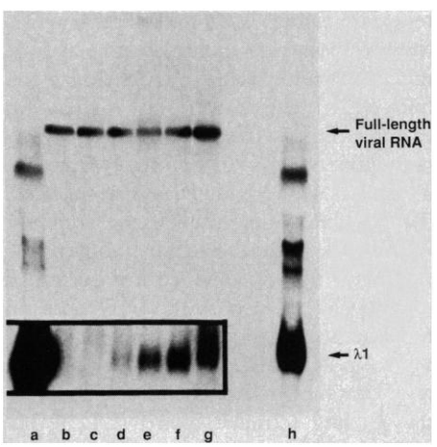

Fig. 3. Tissue-specific expression of the λ1 immunoglobulin gene transferred by infection with the pSVXλ1 8 vector. NIH 3T3, EL4, 1881Y, 70Z, and X63.Ag8.563 cells were infected with virus from ψ2 pSVXλ1 8.3 or ψ2 pSVXλ1 8.6. Infected cells were selected in G418-containing medium. Total cellular RNA (30 μg) from each infected cell line and from the B18 λ1-producing hybridoma was analyzed for the presence of λ1 mRNA by Northern blot analysis as in Fig. 2. After hybridization with a ^{32}P-labeled 4.4-kb λ1 gene probe, full-length viral RNA was detected after a 3-hour exposure of the film, whereas a 72-hour exposure (inset) was required to detect the virally encoded λ1 mRNA. Total cellular RNA from lane a, B18; lane b, NIH 3T3 cells with 8.3; lane c, NIH 3T3 cells with 8.6; lane d, EL4 cells with 8.6; lane e, 1881Y cells with 8.6; lane f, 70Z cells with 8.6; lane g, X63.Ag8.563 cells with 8.6; and lane h, λ1 mRNA from B18.

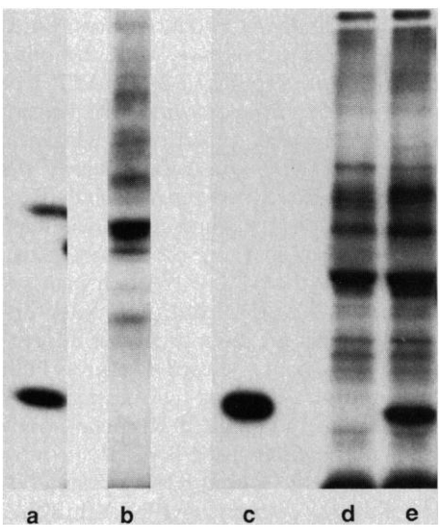

Fig. 4. λ1 light-chain protein is expressed only in B cells having reduced virus transcription as a consequence of infection with a retroviral vector that lacked enhancer sequences. In one experiment (lanes a and b) G418-resistant pSVXλ1-infected X63.Ag8.653 cells were assayed for λ1 light-chain protein by incubating 1×10^6 cells with 100 μCi of [^{35}S]methionine for 3 hours at 37°C (*14*). The resulting detergent extracts were subjected to affinity purification with a monoclonal antibody to Cλ1 (LS 136) (*15*) coupled to Sepharose 4B beads. Protein eluted from the beads was electrophoresed on a 10% polyacrylamide slab gel with a 4.75% stacking gel (*16*). Gels were fixed and impregnated with scintillators as described (*17*) and exposed to Kodak XAR film with DuPont Lightning-Plus intensifying screens. No light-chain protein was seen in the region where authentic ^{125}I-labeled (*18*) λ1 protein (from myeloma HOPC2020) was found (M1) (lane a). In another experiment, X63.Ag8.653 cells were nonselectively infected with pSVXλ1en$^-$ (lane e) or left uninfected (lane d). pSVXλ1en$^-$ is identical to pSVXλ1 except that the 3′ enhancer element has been deleted, from Pvu II to Xba I. Reverse transcription of viral RNA from this vector results in a provirus from which both enhancers have been deleted. A more detailed description of this virus has been reported elsewhere (*6*). Steady-state viral RNA levels are about 1/15 as much in pSVXλ1en$^-$-infected cells as the levels in pSVXλ1-infected cells. A protein comigrating with authentic ^{125}I-labeled λ1 protein (M2) (lane c) is readily detectable after infection of X63.Ag8.653 with pSVXλ1en$^-$.

REFERENCES AND NOTES

1. D. Picard and W. Schaffner, *Nature (London)* **307**, 80 (1984); E. B. Reilly, unpublished results.
2. K. Shimotohno and H. M. Temin, *Cell* **26**, 67 (1981); M. Emerman and H. M. Temin, *J. Virol.* **50**, 42 (1984); A. L. Joyner and A. Bernstein, *Mol. Cell. Biol.* **3**, 2180 (1983).
3. C. L. Cepko *et al.*, *Cell* **37**, 1053 (1984).
4. R. Mann, R. C. Mulligan, D. Baltimore, *ibid.* **33**, 153 (1983).
5. J. G. Izant and H. Weintraub, *ibid.* **36**, 1007 (1984); S. K. Kim and B. J. Wold, *ibid.* **42**, 129 (1985).
6. R. Cone *et al.*, *Mol. Cell. Biol.* **7**, 887 (1987).
7. O. Bernard, N. Hozumi, S. Tonegawa, *Cell* **15**, 1133 (1978).
8. G. J. Todaro and H. Green, *J. Cell Biol.* **17**, 299 (1963).
9. E. J. Siden, D. Baltimore, D. Clark, N. E. Rosenberg, *Cell* **16**, 389 (1979).
10. C. J. Paige, P. W. Kincaide, P. J. Ralph, *J. Immunol.* **121**, 641 (1978).
11. A. W. Harris, A. D. Bankhurst, S. Mason, N. L. Warner, *ibid.* **110**, 431 (1973).
12. K. Horibata and A. W. Harris, *Exp. Cell Res.* **60**, 61 (1970).
13. J. M. Chirgwin *et al.*, *Biochemistry* **18**, 5294 (1979).
14. E. B. Reilly, A. R. Frackelton, Jr., H. N. Eisen, *Eur. J. Immunol.* **12**, 552 (1982).
15. M. Reth, T. Imanishi-Kari, K. Rajewsky, *ibid.* **9**, 1004 (1979).
16. U. K. Laemmli, *Nature (London)* **227**, 680 (1970).
17. W. M. Bonner and R. A. Laskey, *Eur. J. Biochem.* **46**, 83 (1974).
18. D. Haustein, J. J. Marchalonis, A. Harris, *Biochemistry* **12**, 1130 (1973).
19. The 7.4-kb Eco RI fragment encoding the rearranged λ1 gene from HOPC2020 was a gift from B. Blomberg; monoclonal antibody to Cλ1 (LS 136) was a gift from T. Imanishi-Kari. Supported by grants from NIH (CA26712 and CA38497) and the MacArthur Foundation to R.C.M.

13 August 1986; accepted 26 February 1987

Problems in the Use of Survey Questions to Measure Public Opinion

HOWARD SCHUMAN AND JACQUELINE SCOTT

Sample interview surveys are frequently proposed and sometimes used as a way of studying public choices among alternatives. Questions in such surveys may be either "open" or "closed." Two experiments are reported that demonstrate the difficulty of inferring not only absolute levels but even relative orderings of public choices from either type of question, although such questions can be used more successfully to study temporal change or variations across social categories.

A SEEMINGLY SIMPLE WAY OF ASSESSing public opinion is to ask a random sample of the public to choose from among an explicit or implicit set of alternatives. The form of the question, however, can greatly affect such choices. One crucial distinction is whether respondents are expected to answer in their own words from alternatives they construct (open questions) or to select instead from a list of offered alternatives (closed questions). Very little research has been carried out on what effect this difference in question form makes in studying public opinion (*1*).

We present experimental evidence on the limitations of both open and closed questions in attempts to measure public choices. Closed questions are shown to sharply restrict frames of reference by focusing attention on the alternatives offered, no matter how impoverished those alternatives may be and no matter how much effort is made to offer respondents freedom to depart from them. Open questions are shown to exercise their own form of constraint, though in subtle ways that can easily be missed by investigators. The goal of the experiments is not to argue against either form of question, but to emphasize that question content is always based, whether recognized or not, on important assumptions about what should be included in respondent frames of reference. The unexamined question is not worth asking.

Limitations of closed questions. For this experiment we employed a frequently used open question, that about "the most important problem facing this country today" (Table 1) (*2*). This open question was asked to a random half of a national sample in the October 1986 Monthly Random Digit Dial Telephone Survey conducted by the Survey Research Center. The other half of the sample was asked a specially constructed closed version of the question (Table 1). The closed version listed four problems, each of which had been mentioned by less than 1% of the population in recent use of the open question by the Gallup organization. Respondents were not, however, forced to choose one of these rare alternatives, but were told as part of the question that "if you prefer, you may name a different problem as most important."

As expected, Table 1 shows that less than 3% of the national sample spontaneously mentioned any of the four "rare" problems to the open question. The categories most frequently coded were unemployment (17%), general economic problems (17%), threat of nuclear war (12%), and foreign affairs (10%), with the rest of the responses scattered among a dozen categories, including 5% "don't know."

On the closed form, however, 60% of the sample chose one of the four "rare" alternatives as "most important," only 40% taking the option of naming some other problem. Moreover, unemployment, the most frequently mentioned single problem on the open form, was given by only 6.2% of the respondents on the closed form.

On the basis of the closed question, one would conclude that the quality of public schools is what troubles Americans most, followed by the issue of pollution and then by abortion, whereas on the open question it is economic and international problems that loom largest, while the issues of education, pollution, and abortion are practically invisible.

Most readers will assume, as do we, that the issues mentioned on the open question give the better overall picture of American concerns and that the findings on the closed question are distorted by the constraint or inertia produced by listing the four problems as part of the question, despite the explicit provision offered to respondents to depart from them (*3*).

The limitations of open questions. The preceding results suggest that open questions provide a clearer picture of the concerns of a survey sample than do closed questions. Yet this ignores the possibility that open questions can also provide a constraining frame of reference. The following experiment was carried out to test this assumption as clearly as possible.

The experiment was suggested in the course of another survey. Respondents had been asked to name one or two of the most important "national or world event (events) or change (changes)" during the past 50 years that came to mind. To this open question, the most commonly given responses had to do with World War II and the Vietnam War, but, as intended, many answers referred to broader social changes, such as the civil rights movement or to scientific and technological developments, such as space exploration. Hardly mentioned at all, however, was the development of the computer, which might not have seemed surprising except that references to computers occurred frequently in responses to later questions.

This discrepancy suggested that computers had made a considerable impact on the public, but that the "national or world event or change" open question tended unwitting-

Survey Research Center, Institute for Social Research, University of Michigan, Ann Arbor, MI 48106-1248.