JOURNAL OF VIROLOGY, Apr. 1993, p. 1811–1816
0022-538X/93/041811-06$02.00/0

Vol. 67, No. 4

Isolation of a Chicken Gene That Confers Susceptibility to Infection by Subgroup A Avian Leukosis and Sarcoma Viruses

JOHN A. T. YOUNG,[1]†* PAUL BATES,[1] AND HAROLD E. VARMUS[1,2]

Department of Microbiology and Immunology[1] and Department of Biochemistry and Biophysics,[2] University of California School of Medicine, San Francisco, California 94143

Received 2 October 1992/Accepted 21 December 1992

We used a genetic strategy to isolate the chicken gene believed to encode the receptor for subgroup A avian leukosis and sarcoma viruses (ALSV-A). Chicken genomic DNA was transfected into monkey COS-7 cells, and two independent primary transfectants susceptible to ALSV-A infection were identified by using ALSV-A vectors containing a hygromycin B resistance gene. A second round of transfection and selection in mouse BALB/3T3 fibroblasts again led to isolation of a transfectant susceptible to infection by ALSV-A. Plasmid DNA sequences linked to chicken DNA during the primary transfection segregated with chicken DNA in the secondary transfectant and served as a molecular tag to clone the gene conferring susceptibility. Expression of the cloned gene in mouse BALB/3T3 cells conferred susceptibility to infection by ALSV-A but not by ALSV-B. Therefore the cloned gene most probably represents the *tv-a* locus, the genetically defined receptor gene for ALSV-A.

Retroviruses recognize and infect target cells that produce virus-specific cell surface receptors (31). The interactions between viral envelope (Env) glycoproteins and host cell surface receptors lead to fusion of virus and cell membranes, so that viral nucleoprotein core particles are introduced into the host cell cytoplasm. However, these early events in the virus life cycle are poorly understood.

The interactions between avian leukosis and sarcoma virus (ALSV) Env proteins and cellular receptors provide a useful model system for understanding how retroviruses infect cells. There are five major subgroups of ALSV (designated A to E). Viruses within each subgroup have the same host range, encode immunologically related envelope (Env) glycoproteins, and demonstrate cross-interference (6, 10, 13, 20, 27–29).

Chickens have been classified by their susceptibility to different ALSV subgroups. For example, C/O chickens can be infected by all five major virus subgroups, whereas C/AB lines are resistant to infection by ALSV-A and ALSV-B (27). ALSV-A can bind equally well to genetically resistant (C/A) and susceptible (C/O) cells, leading to the suggestion that subgroup-specific ALSV susceptibility factors function at the level of virus penetration rather than virus adsorption to target cell surfaces (23).

Genetic studies have identified three autosomal chicken loci, designated *tv-a*, *tv-b*, and *tv-c*, which govern susceptibility to infection by ALSV-A, ALSV-B, and ALSV-C, respectively (4, 20–22, 24). These loci are presumed to encode subgroup-specific virus receptors. ALSV-B, ALSV-D, and ALSV-E exhibit some degree of cross-interference, suggesting that receptors for these virus subgroups are encoded by particular alleles at the *tv-b* locus (31).

Since ALSV receptors are each the product of a single genetic locus, we predicted that the genes encoding these susceptibility factors could be isolated by a gene transfer strategy. We describe the use of this approach to isolate a chicken gene which appears to encode the ALSV-A receptor, since expression of this gene in mammalian cells confers susceptibility to infection specifically by ALSV-A.

* Corresponding author.
† Present address: Gladstone Institute of Virology and Immunology, P.O. Box 419100, San Francisco, CA 94141.

MATERIALS AND METHODS

Plasmids, cells, viruses, and DNA transfections. Plasmid pMPneo conferring resistance to G418 was provided by M. Scott. Plasmid pMPHis was constructed by replacing the G418^r gene of pMPneo with the bacterial histidinol dehydrogenase gene contained on a 1.35-kb *Bam*HI fragment (11). Monkey COS-7 cells and quail QT6 cells were described previously (9, 18). BALB/3T3 cells (clone A31) were provided by D. Kaplan. Chicken embryo fibroblasts were prepared either from C/O (chf$^-$) embryos from SPAFAS or from line O embryos from U.S. Department of Agriculture Regional Poultry Laboratories. A hygromycin B resistance gene was introduced into the *Cla*I cloning site of either the ALSV-A vector RCAS or an ALSV-B vector (12), generating constructs RCASH-A and RCASH-B, respectively. Similarly, a methotrexate resistance allele of the mouse dihydrofolate reductase gene driven by a simian virus 40 early promoter (25) was introduced into the *Cla*I site of the RCAS vector, generating the RCASM-A virus. Cells were transfected with DNA by calcium phosphate precipitation (32). The ALSV vectors were introduced by transfection into chicken fibroblasts, and the cells were passaged to allow virus spread. Cells infected by RCASH-A and RCASH-B viruses were selected by using 80 μg of hygromycin B per ml. Virus stocks prepared from confluent plates of these cells were filtered through 0.2-μm-pore-size filters and stored at −70°C. By using QT6 cells as recipients, the titers of RCASH-A were 10^4 to 10^5 hygromycin B-resistant CFU/ml. Relative titers of RCASH-A and RCASH-B were obtained by serially diluting each virus stock and infecting C/O chicken embryo fibroblasts at 20% confluence on 60-mm plates. The chicken cells were grown to confluence after infection and split onto a new plate for selection in hygromycin B (80 μg/ml). The titer of RCASH-A was estimated to be one-fifth as high as that of RCASH-B by comparing the

overall numbers of hygromycin B-resistant cells after serially diluting each virus stock.

Primary transfection. A total of 5×10^5 COS-7 cells were plated on each of 30 100-mm plates. Each plate of cells was transfected with 20 μg of chicken genomic DNA plus 1 μg of linearized pMPneo plasmid DNA. After 48 h, cells were selected by using 300 μg of G418 per ml. Approximately 50 to 100 G418-resistant colonies were detected on each plate and were grown to an average size of 200 cells per colony. The cells on each plate were then split onto duplicate plates and infected three times during a 24-h period with approximately 10^4 hygromycin-resistant CFU of RCASH-A virus (titered on QT6 cells) per plate. Virus-infected cells were selected by using hygromycin B at 300 μg/ml.

Secondary transfection. A total of 5×10^5 BALB/3T3 cells were plated on each of 80 100-mm plates, and each plate was transfected with 18.5 μg of genomic DNA from colony H representing primary transfectant no. 2 and 2 μg of pmpHis plasmid DNA. After 48 h, cells were selected in medium lacking histidine and containing 200 μM histidinol. Five days later the cells on each plate were split onto duplicate plates, subconfluent cells were exposed three times over a 24-h period to 10^4 hygromycin B-resistant CFU of RCASH-A virus per plate, and infected cells were selected by using hygromycin B at 300 μg/ml. The identity of ALSV receptor-expressing cells was confirmed by reinfecting with RCASM-A viruses, which conferred resistance to 250 nM methotrexate.

Southern blot analysis. Approximately 10 μg of genomic DNA was digested with restriction enzymes, electrophoresed on 1% agarose gels, transferred to Hybond-N (Amersham) nylon filters, and incubated with radioactively labeled DNA probes as described previously (3). Radioactively labeled probes were prepared by random oligonucleotide priming (7). Southern blots were exposed for autoradiography at −70°C with Kodak XAR-5 film and intensifying screens. The ALSV *gag*-specific probe was a 1,384-bp *Bam*HI fragment representing nucleotides 532 to 1916 of the ALSV genome. The *neo* probe was a 1.4-kb fragment representing the entire coding region of the gene conferring resistance to G418. The ALSV receptor locus probe was a 550-bp *Hin*cII-*Eco*RI fragment derived from λ1 (see Fig. 4).

Isolation of genomic DNA clones. A size-selected (11- to 15-kb) library of *Eco*RI-digested fragments from the genomic DNA of the secondary transfectant was constructed by using λDASHII (Stratagene). Clone λ1 was isolated from the library by using the radioactively labeled *neo* hybridization probe. A library of partially digested *Eag*I fragments of C/O chicken genomic DNA was constructed in λDASHII. Clone λ2 was isolated from this library by using the radioactively labeled *Hin*cII-*Eco*RI DNA fragment of λ1 as a hybridization probe.

Virus infection of mouse cells. A total of 1.5×10^5 mouse BALB/3T3 cells were plated on 60-mm plates and transfected with 0.5 μg of pmpHis DNA and 4 μg of λ1, 4 μg of λ2, or 2 μg of λ1 plus 2 μg of λ2. Cells were selected in medium lacking histidine and containing 200 μM histidinol, yielding approximately 30 histidinol-resistant colonies on each plate. These populations of cells were incubated with either RCASH-A or RCASH-B viruses for 24 h and selected in medium containing 300 μg of hygromycin B per ml.

RESULTS

Primary and secondary transfer of chicken DNA conferring susceptibility to ALSV-A infection upon mammalian cells. Genomic DNA from C/O chicken embryo fibroblasts was transfected into monkey COS-7 cells along with plasmid DNA conferring resistance to G418. COS-7 cells were chosen as recipients because they are normally resistant to ALSV-A but appear to have no intracellular block to ALSV provirus formation since they are efficiently infected by mammal-tropic ALSV-D (2). To identify transfected cells that could be infected by ALSV-A, G418-resistant colonies were split onto duplicate plates and challenged with ALSV-A-specific vectors (designated RCASH-A) containing a hygromycin B resistance gene. Transfected cells, containing a chicken gene that confers susceptibility to ALSV-A infection, should have given rise to multiple independent hygromycin B-resistant colonies on both duplicate plates (Fig. 1). In contrast, nonspecific infection of other transfected cells would at most result in one or two hygromycin B-resistant colonies (Fig. 1).

Multiple hygromycin B-resistant colonies were detected on a pair of duplicate plates and on a third independent plate. We used several different criteria to confirm that colonies on these plates contain a chicken gene that confers susceptibility to infection by ALSV-A. First, the multiple colonies on each plate should be derived from the progeny of a single primary transfectant that was expanded from the original plate onto duplicate plates before virus infection (Fig. 1). Therefore, multiple colonies on each plate should contain an identical pattern of transfected DNA sequences. This was demonstrated by Southern blot analysis of genomic DNA prepared from individual colonies by using radioactively labeled plasmid DNA sequences as a hybridization probe; four colonies (A to D) from the single plate and four colonies (E to H) from the pair of duplicate plates contained the same transfected DNA sequences (Fig. 2, left panel). Second, each hygromycin B-resistant colony should have resulted from independent virus infections so that each colony should contain at least one RCASH-A provirus, all integrated at different sites in the genome. This was tested by Southern blot analysis by using a radioactively labeled ALSV *gag*-specific probe to detect restriction enzyme site variation in the genomic DNA that flanks the 5′ end of each provirus. The sizes of restriction fragments detected by this probe differed between individual colonies (Fig. 2, right panel), confirming that each of these colonies was the result of independent virus infections. Furthermore, these cells had been efficiently infected since they generally contained more than one RCASH-A provirus (Fig. 2, right panel; Table 1). Apparently, superinfection resistance had not been established in these cells since they could be efficiently reinfected by ALSV-A that contained either a diagnostic polymorphic restriction enzyme site or a mutant dihydrofolate reductase gene that confers resistance to methotrexate (data not shown). Taken together, these observations confirmed that we had isolated two independent cell lines rendered susceptible to infection by ALSV-A as a result of the introducing chicken genomic DNA by transfection.

Southern blot analysis with a radioactively labeled probe specific for chicken repeat DNA sequences revealed that both primary transfectants contained multiple fragments of chicken genomic DNA (data not shown). To segregate the ALSV-A susceptibility gene from the other chicken DNA sequences in these cells, we performed a second round of transfection and selection. Genomic DNA from a primary transfectant (colony H in Fig. 2) was transfected into mouse BALB/3T3 cells along with plasmid DNA conferring resistance to histidinol (see Materials and Methods). Histidinol-resistant transfectants were split onto duplicate plates, challenged with RCASH-A viruses containing a hygromycin B resistance gene as above, and selected by using hygromycin

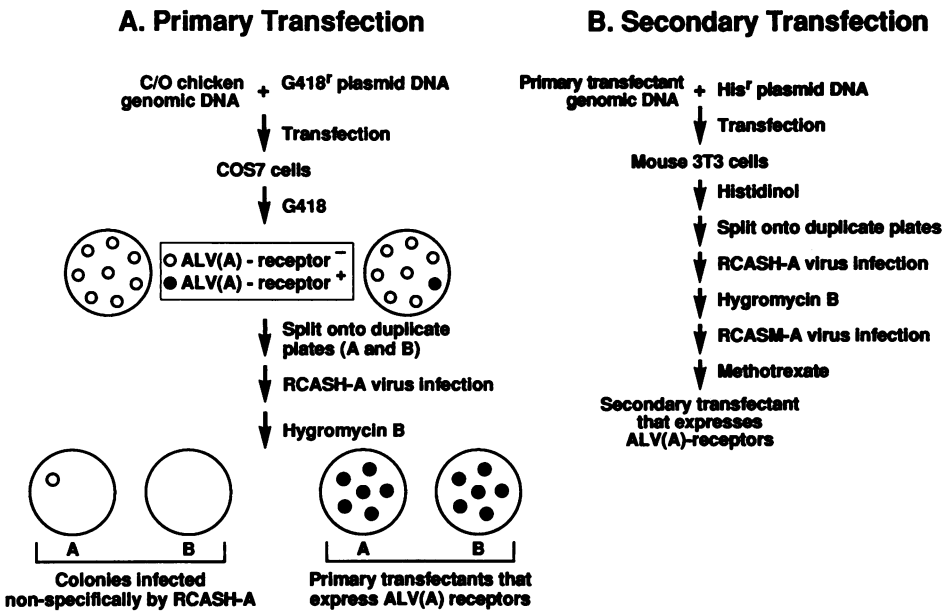

FIG. 1. Genetic strategy to isolate the chicken ALSV-A receptor gene.

B (Fig. 1). In this protocol, cells could be hygromycin B resistant either because they acquired the susceptibility gene in the secondary transfection and were infected by RCASH-A viruses or because they had been transfected with an RCASH-A provirus contained in the primary transfectant DNA. To distinguish between these two possibilities, hygromycin B-resistant cells were also challenged with the RCASM-A virus, containing a gene conferring resistance to methotrexate. Three colonies (A to C) derived from one plate of cells were resistant to methotrexate. Southern blot analysis of genomic DNA isolated from each of these colonies (before they were infected with RCASM-A virus) revealed that they

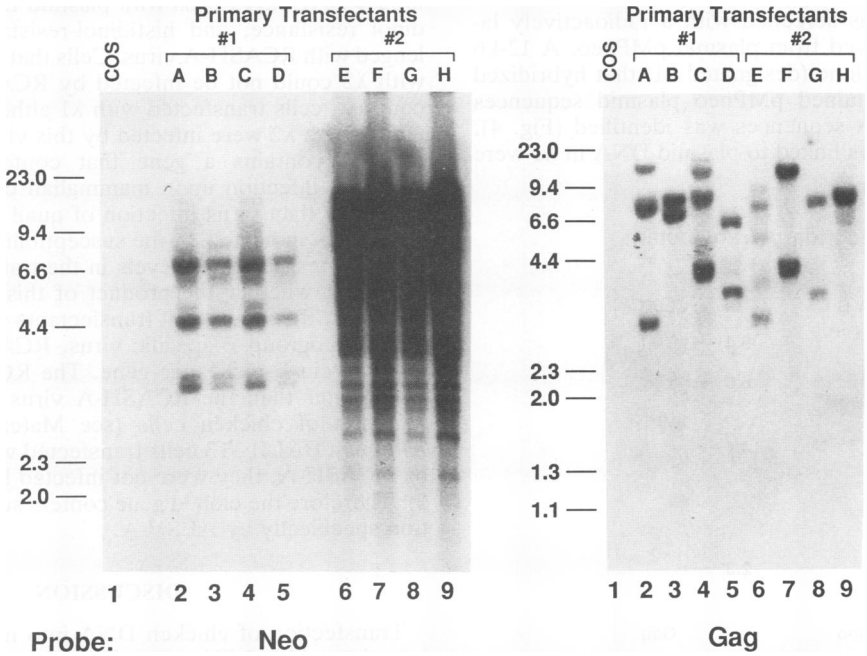

FIG. 2. Analysis of genomic DNA from virus-infected primary transfectants. Genomic DNA was prepared from colonies infected by RCASH-A virus after the primary transfection. Colonies A to D (lanes 2 to 5) were derived from the same plate of cells, and colonies E to F (lanes 6 to 9) were from a pair of duplicate plates as described in the text. Lane 1 in each panel contains DNA from untransfected COS-7 cells. (Left) *Bcl*I-digested samples were hybridized with the radioactively labeled *neo* probe. *Bcl*I cuts once in the G418^r gene of the transfected plasmid sequences. (Right) *Hin*dIII-digested samples were hybridized with the radioactively labeled ALSV *gag*-specific probe to detect different proviral integration sites; *Hin*dIII cuts in the viral *pol* gene and in the genomic DNA flanking the 5′ end of each provirus. DNA size markers are in kilobases.

TABLE 1. Two independent primary transfectants that express receptors for ALSV-A[a]

Primary transfectant	Colony	Estimated no. of copies of G418[r] plasmid DNA	Estimated no. of RCASH-A proviruses
1	A	4	4
	B	4	2
	C	4	6
	D	4	5
2	E	50	7
	F	50	3
	G	50	2
	H	50	1

[a] These data summarize the results of Southern blot analysis from Fig. 2.

all contained the same transfected DNA sequences (Fig. 3, left panel). These cells had been efficiently infected since they contained several RCASH-A proviruses at different positions in the genome (Fig. 3, right panel). We concluded that these colonies were derived from a single cell that acquired a chicken gene conferring susceptibility to ALSV-A infection in the secondary transfection.

Isolation of chicken genomic DNA sequences linked to the ALSV-A susceptibility locus. Analysis of restriction digests of DNA from primary and secondary transfectants indicated that a single copy of pMPneo plasmid sequences contained in primary transfectant no. 2 segregated with an ALSV-A susceptibility gene during the second round of transfection (Fig. 3, left panel). This suggested that plasmid DNA was linked to the susceptibility gene and, if so, might serve as a molecular tag to clone the gene. To test this, a lambda phage library of genomic DNA sequences prepared from the secondary transfectant was screened with a radioactively labeled DNA probe derived from plasmid pMPneo. A 12-kb *Eco*RI genomic DNA clone (designated λ1) that hybridized to the probe and contained pMPneo plasmid sequences linked to chicken DNA sequences was identified (Fig. 4). Chicken DNA sequences linked to plasmid DNA in λ1 were

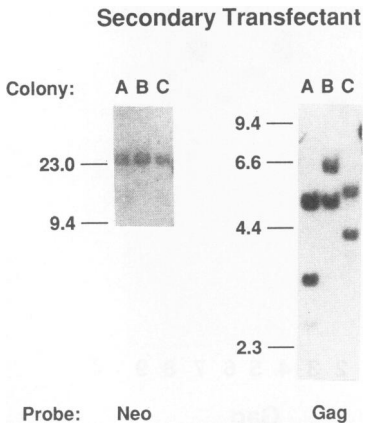

FIG. 3. Analysis of genomic DNA from virus-infected secondary transfectants. Genomic DNA was prepared from three colonies (A to C) that resulted from infection by both RCASH-A and RCASM-A viruses after secondary transfection with DNA from primary transfectant no. 2. *Hin*dIII-digested samples were hybridized either with the radioactively labeled *neo* probe (left) or with the ALSV *gag*-specific probe (right). DNA size markers are in kilobases.

contained in both independent primary transfectants (Fig. 4). Therefore, these sequences consistently cosegregated with the ALSV-A susceptibility gene and must be linked to this gene. To isolate additional sequences from this locus, a radioactively labeled probe from λ1 was used to isolate an overlapping 7-kb *Eag*I genomic DNA fragment, designated λ2, from a chicken genomic DNA library (Fig. 4). By comparing restriction enzyme maps of this locus in the primary and secondary transfectants, the ALSV-A susceptibility gene was localized to a fragment contained within approximately 7 kb of the plasmid DNA sequences in λ1. The differences in positions of *Hin*dIII restriction enzyme sites to the right of the plasmid DNA (Fig. 4) define the breakpoint boundaries beyond which the sequences in the secondary transfectant differ from those in the primary transfectant.

Two functional alleles of the ALSV-A susceptibility gene. A radioactively labeled DNA probe derived from single-copy chicken DNA in λ1 (Fig. 4) was used for Southern blot analysis of genomic DNA samples digested with *Eco*RI and *Bam*HI. This probe detected two fragments (1.6 and 2.1 kb) in the chicken DNA used for transfection (Fig. 5, lane 1). The difference in size between these two fragments is due to a polymorphic *Eco*RI restriction enzyme site (indicated by an asterisk in Fig. 4) that distinguishes two different alleles at this locus. Apparently each of these alleles had been independently transfected into the two primary transfectants that contain an ALSV-A susceptibility gene (Fig. 5, lanes 2 to 5). We conclude from these findings that both alleles of this gene confer susceptibility to ALSV-A.

The cloned gene confers susceptibility to ALSV-A but not ALSV-B. Since the ALSV-A susceptibility gene was localized to a 7-kb chicken DNA fragment, we next determined whether genomic clone λ1 or λ2 could confer susceptibility to RCASH-A infection. The clones were transfected into mouse BALB/3T3 cells with plasmid DNA conferring histidinol resistance, and histidinol-resistant cells were challenged with RCASH-A virus. Cells that had been transfected with λ2 could not be infected by RCASH-A (Table 2). In contrast, cells transfected with λ1 either alone or in combination with λ2 were infected by this virus (Table 2). Therefore λ1 contains a gene that confers susceptibility to ALSV-A infection upon mammalian cells, albeit with less efficiency than virus infection of quail QT6 cells (Table 2). This might indicate that the susceptibility factor encoded by λ1 is expressed at low levels in the transfected cells.

To test whether the product of this gene is specific for ALSV-A, the mouse cell transfectants were also challenged with a subgroup B-specific virus, RCASH-B, containing a hygromycin B-resistance gene. The RCASH-B virus had a higher titer than the RCASH-A virus when compared by infection of chicken cells (see Materials and Methods). Although BALB/3T3 cells transfected with λ1 were infected by RCASH-A, they were not infected by RCASH-B (Table 2). Therefore the cloned gene confers susceptibility to infection specifically by ALSV-A.

DISCUSSION

Transfection of chicken DNA into mammalian cells and infection with ALSV-A that contain selectable genes led to the isolation of an avian genomic DNA clone that confers susceptibility to infection by ALSV-A but not by ALSV-B. Since the subgroup specificity of these viruses maps to the viral surface Env protein (2, 5) and operates at the level of virus entry into cells, we draw the tentative conclusion that the isolated gene is *tv-a*, the genetically defined receptor

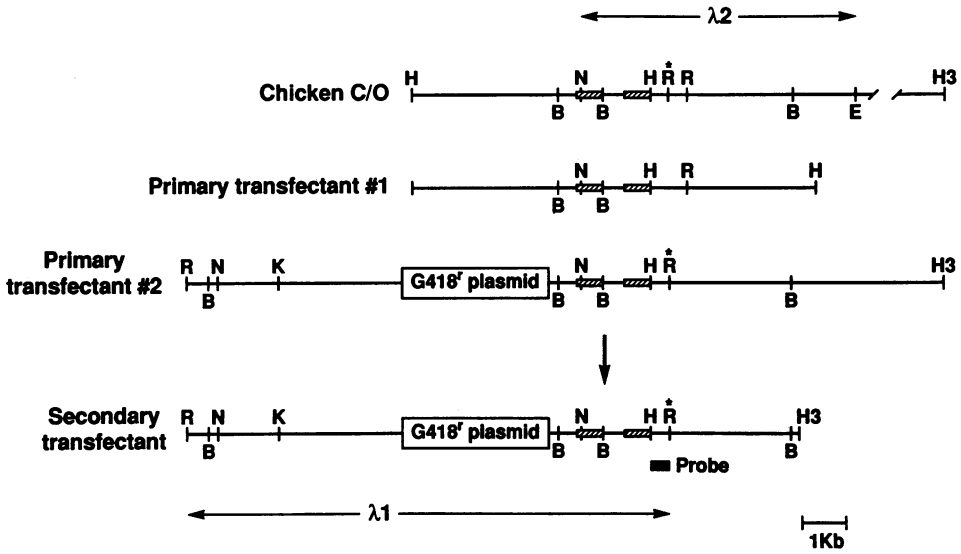

FIG. 4. The ALSV-A susceptibility locus in chicken cells and in transfected cells. A 12-kb *Eco*RI fragment (λ1; bottom), which contained both plasmid and linked chicken DNA sequences, was cloned from the secondary transfectant. An overlapping 7-kb *Eag*I fragment (λ2; top) was subsequently cloned from chicken cells. Hatched boxes represent CR1 chicken repeat DNA sequences (26). Restriction enzymes: B, *Bam*HI; N, *Not*I (contains a recognition site for *Eag*I); H, *Hin*cII; R, *Eco*RI; R*, polymorphic *Eco*RI site (see Fig. 5); E, *Eag*I; H3, *Hin*dIII. The ALSV-A susceptibility gene contains at least three other *Eag*I sites not indicated here. The solid box indicates a 550-bp *Eco*RI-*Hin*cII fragment that was used as a radioactively labeled DNA probe in the experiments in Fig. 5.

locus for ALSV-A. Additional experiments are required to determine the identity of this gene. If the cloned gene is *tv-a*, radioactively labeled probes derived from this locus may identify characteristic restriction fragment length polymorphisms associated with susceptible (*tv-a*^{s}) or resistant (*tv-a*^{r}) alleles in different chicken strains. Also, alleles of the cloned gene isolated from genetically resistant chicken strains would not be expected to confer susceptibility to ALSV-A.

Southern blot analysis with several independent radioactively labeled fragments of genomic clone λ1 as probes, identified the ALSV-A susceptibility locus in genomic DNA from turkey and quail cells (1a). Since these fragments are conserved between species, we assumed that they contain coding regions of the ALSV-A susceptibility gene. However, using these probes for Northern blot analysis, we could not detect mRNA transcripts from chicken embryo fibroblasts and could not identify any cDNA clones in several libraries prepared from chicken cells. Also, the primary amino acid sequence of the ALSV-A susceptibility factor could not be deduced by DNA sequence analysis of these fragments since each fragment contained more than one possible open reading frame. For these reasons, we decided to isolate the quail homolog of the chicken gene so that this locus could be compared between species. We have now used a retrovirus-based exon-trapping approach to identify the domains of the quail ALSV-A susceptibility gene that encode a functional virus receptor (1a).

Like most other retroviruses, ALSVs infect cells in a pH-

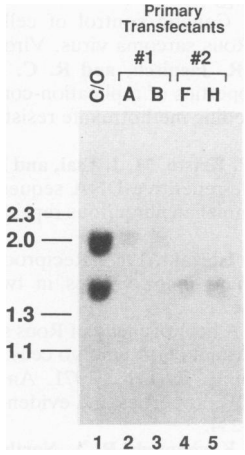

FIG. 5. Two functional alleles of the ALSV-A susceptibility gene. Genomic DNA from C/O chicken cells (lane 1), from colonies A and B derived from primary transfectant no. 1 (lanes 2 and 3), and from colonies F and H representing primary transfectant no. 2 (lanes 4 and 5) were digested with *Eco*RI and *Bam*HI. Samples were incubated with the radioactively labeled 550-bp fragment derived from the ALSV-A susceptibility gene (described in the legend to Fig. 4). DNA size markers are in kilobases.

TABLE 2. Genomic clone λ1 confers susceptibility to ALSV-A infection[a]

Cells used	No. of hygromycin-resistant colonies/ml in expt:			
	1 (RCASH-A)	2 (RCASH-A)	3	
			RCASH-A	RCASH-B
BALB/3T3	0	0	0	0
BALB/3T3 (λ1)	60	7	32	0
BALB/3T3 (λ2)	0	0	0	0
BALB/3T3 (λ1+λ2)	60	80	64	0

[a] Mouse BALB/3T3 cells were stably transfected with genomic clone λ1 or λ2 (or λ1 and λ2) and grown as pools of cells. In experiments 1 and 2, 2×10^5 cells were incubated with RCASH-A virus (approximately 10^4 hygromycin B-resistant QT6 colonies per ml). In experiment 3, 5×10^4 cells were incubated either with RCASH-A virus or with a higher-titer stock of RCASH-B virus as described in Materials and Methods.

independent manner, suggesting that the virus membrane fuses directly with the host cell surface membrane (8, 17). Interestingly, subgroup-specific ALSV susceptibility factors may function to mediate virus penetration rather than adsorption at the target cell surface (23). Future experiments will determine whether the susceptibility factor encoded by the chicken gene we have cloned mediates virus penetration. If so, the function of this factor in virus entry may be fundamentally different from that performed by the human CD4 receptor. Human CD4 produced in mouse cells can bind human immunodeficiency virus type 1, but this interaction does not appear to be sufficient to allow virus entry (16).

Retroviruses have evolved to use proteins with very different structures and functions as cellular receptors. CD4, the receptor for human and simian immunodeficiency viruses, is a member of the immunoglobulin superfamily and normally functions in T-cell signaling (16). In contrast, receptors for ecotropic murine leukemia viruses and gibbon ape leukemia viruses are proteins predicted to have multiple membrane-spanning domains (1, 19). The ecotropic murine leukemia virus receptor is a cationic amino acid transporter (15, 30), and the gibbon ape leukemia virus receptor bears structural similarity to a phosphate transporter (14). Further study of the gene we have described in this report may help determine whether these receptors share some more subtle feature that is essential for their function in retrovirus entry. Alternatively, there might be other, more highly conserved susceptibility factors in each of these systems that act in concert with the identified receptors to allow virus infection.

ACKNOWLEDGMENTS

We thank Mark Feinberg for critically reading the manuscript. We also thank S. Hughes, S. Hartman, and H. Stuhlmann for kindly providing reagents and S. Ortiz for preparing primary chicken cells.

This work was performed with a grant from the NIH. J.A.T.Y. was supported by postdoctoral fellowships from EMBO and from the Arthritis Foundation. H.E.V. is an American Cancer Society Research Professor.

REFERENCES

1. **Albritton, L. M., L. Tseng, D. Scadden, and J. M. Cunningham.** 1989. A putative murine ecotropic retrovirus receptor gene encodes a multiple membrane-spanning protein and confers susceptibility to virus infection. Cell **57**:659–666.
1a. **Bates, P., J. Young, and H. E. Varmus.** Unpublished data.
2. **Bova, C. A., J. C. Olsen, and R. Swanstrom.** 1988. The avian retrovirus *env* gene family: molecular analysis of host range and antigenic variants. J. Virol. **62**:75–83.
3. **Church, G. M., and W. Gilbert.** 1984. Genomic sequencing. Proc. Natl. Acad. Sci. USA **81**:1991–1995.
4. **Crittenden, L. B., H. A. Stone, R. H. Reamer, and W. Okazaki.** 1967. Two loci controlling genetic cellular resistance to avian leukosis-sarcoma viruses. J. Virol. **1**:898–904.
5. **Dorner, A. J., and J. M. Coffin.** 1986. Determinants for receptor interaction and cell killing on the avian retrovirus glycoprotein gp85. Cell **45**:365–374.
6. **Duff, R. G., and P. K. Vogt.** 1969. Characteristics of two new avian tumor virus subgroups. Virology **39**:18–30.
7. **Feinberg, A. P., and B. Vogelstein.** 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132**:6–13.
8. **Gilbert, J. M., D. Mason and J. M. White.** 1990. Fusion of Rous sarcoma virus with host cells does not require exposure to low pH. J. Virol. **64**:5106–5113.
9. **Gluzman, Y.** 1981. SV40-transformed simian cells support the replication of early SV40 mutants. Cell **23**:175–182.
10. **Hanafusa, H.** 1965. Analysis of the defectiveness of Rous sarcoma virus. III. Determining influence of a new helper virus on the host range and susceptibility to interference of RSV. Virology **25**:248–255.
11. **Hartman, S. C., and R. C. Mulligan.** 1988. Two dominant-acting selectable markers for gene transfer studies in mammalian cells. Proc. Natl. Acad. Sci. USA **85**:8047–8051.
12. **Hughes, S. H., J. J. Greenhouse, C. J. Petropoulos, and P. Sutrave.** 1987. Adaptor plasmids simplify the insertion of foreign DNA into helper-independent retroviral vectors. J. Virol. **61**: 3004–3012.
13. **Ishizaki, R., and P. K. Vogt.** 1966. Immunological relationships among envelope antigens of avian tumor viruses. Virology **30**:375–387.
14. **Johann, S. V., J. J. Gibbons, and B. O'Hara.** 1992. GLVR1, a receptor for gibbon ape leukemia virus, is homologous to a phosphate permease of *Neurospora crassa* and is expressed at high levels in the brain and thymus. J. Virol. **66**:1635–1640.
15. **Kim, J. W., E. I. Closs, L. M. Albritton, and J. M. Cunningham.** 1991. Transport of cationic amino acids by the mouse ecotropic retrovirus receptor. Nature (London) **352**:725–728.
16. **Maddon, P. J., A. G. Dalgleish, J. S. McDougal, P. R. Clapham, R. A. Weiss, and R. Axel.** 1986. The T4 gene encodes the AIDS virus receptor and is expressed in the immune system and the brain. Cell **47**:333–348.
17. **McClure, M. O., M. A. Sommerfelt, M. Marsh, and R. A. Weiss.** 1990. The pH independence of mammalian retrovirus infection. J. Gen. Virol. **71**:767–773.
18. **Moscovici, C., M. G. Moscovici, H. Jimenez, M. M. C. Lai, M. J. Hayman, and P. K. Vogt.** 1977. Continuous tissue culture cell lines derived from chemically induced tumors of Japanese quail. Cell **11**:95–103.
19. **O'Hara, B., S. V. Johann, H. P. Klinger, D. G. Blair, H. Rubinson, K. J. Dunn, P. Sass, S. M. Vitek, and T. Robins.** 1990. Characterization of a human gene conferring sensitivity to infection by gibbon ape leukemia virus. Cell Growth Differ. **1**:119–127.
20. **Payne, L. N., and P. M. Biggs.** 1964. Differences between highly inbred lines of chickens in the response to Rous sarcoma virus of the chorioallantoic membrane and of embryonic cells in tissue culture. Virology **24**:610–616.
21. **Payne, L. N., and P. M. Biggs.** 1966. Genetic basis of cellular susceptibility to the Schmidt-Ruppin and Harris strains of Rous sarcoma virus. Virology **29**:190–198.
22. **Payne, L. N., and P. K. Pani.** 1971. Evidence for linkage between genetic loci controlling response of fowl to subgroup A and subgroup C sarcoma viruses. J. Gen. Virol. **13**:253–259.
23. **Piraino, F.** 1967. The mechanism of genetic resistance of chick embryo cells to infection by Rous sarcoma virus-Bryan strain (BS-RSV). Virology **32**:700–707.
24. **Rubin, H.** 1965. Genetic control of cellular susceptibility to pseudotypes of Rous sarcoma virus. Virology **26**:270–276.
25. **Stuhlmann, H., R. Jaenisch, and R. C. Mulligan.** 1989. Construction and properties of replication-competent murine retroviral vectors encoding methotrexate resistance. Mol. Cell. Biol. **9**:100–108.
26. **Stumph, W. E., P. Kristo, M. J. Tsai, and B. W. O'Malley.** 1981. A chicken middle-repetitive DNA sequence which shares homology with mammalian ubiquitous repeats. Nucleic Acids Res. **9**:5383–5397.
27. **Vogt, P., and R. Ishizaki.** 1965. Reciprocal patterns of genetic resistance to avian tumor viruses in two lines of chickens. Virology **26**:664–672.
28. **Vogt, P. K.** 1965. A heterogeneity of Rous sarcoma virus revealed by selectively resistant chick embryo cells. Virology **25**:237–247.
29. **Vogt, P. K., and R. R. Friis.** 1971. An avian leukosis virus related to RSV(O). Properties and evidence for helper activity. Virology **43**:223–234.
30. **Wang, H., M. P. Kavanaugh, R. A. North, and D. Kabat.** 1991. Cell surface receptor for ecotropic murine retroviruses is a basic amino acid transporter. Nature (London) **352**:729–731.
31. **Weiss, R.** Cellular receptors and viral glycoproteins involved in retrovirus entry. *In* J. A. Levy (ed.), The retroviruses, vol. 2, in press. Plenum Press, New York.
32. **Wigler, M., S. Silverstein, L. S. Lee, A. Pellicer, Y. Cheng, and R. Axel.** 1977. Transfer of purified herpes virus thymidine kinase gene to cultured mouse cells. Cell **11**:223–232.