Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 6053–6057, August 1986
Genetics

Differences in sequences encoding the carboxyl-terminal domain of the epidermal growth factor receptor correlate with differences in the disease potential of viral *erbB* genes

DANIEL C. GAMETT*, SHARON E. TRACY†, AND HARRIET L. ROBINSON*

*Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545; and †Department of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester, MA 01605

Communicated by Paul C. Zamecnik, April 29, 1986

ABSTRACT **Eleven recently isolated *erbB*-transducing viruses as well as avian erythroblastosis virus (AEV)-R (ES4) and AEV-H have been characterized for the type of disease they cause, their ability to transform fibroblasts in culture, their ability to cause disease in pedigrees of chicken that differ in susceptibility to *erbB*-induced erythroblastosis, and the structure of their *erbB* genes. Differences in each of the biological parameters correlated with differences in *erbB* sequences encoding the C-terminal domain of the epidermal growth factor receptor (EGFR). Seven viruses were strain restricted in their ability to induce erythroblastosis and did not transform fibroblasts. These seven viruses contained v-*erbB* genes encoding the complete C terminus of the EGFR. AEV-R and AEV-H were not pedigree restricted in their ability to induce erythroblastosis and could transform fibroblasts. These viruses contain v-*erbB* genes that lack codons for the immediate C terminus of the EGFR. Three viruses caused angiosarcoma and one caused fibrosarcoma. The angiosarcoma and fibrosarcoma-inducing viruses were not strain restricted and did not cause erythroblastosis. The v-*erbB* genes of each of these viruses contained extensive internal deletions or 3′ truncations in sequences encoding the C-terminal domain of the EGFR.**

The epidermal growth factor receptor (EGFR) contains four known functional domains: an N-terminal, extracellular, epidermal growth factor (EGF)-binding domain, a transmembrane domain, a tyrosine kinase domain, and an ≈20-kDa C-terminal domain containing the three major sites of EGF-stimulated autophosphorylation (1–4). The v-*erbB* oncogenes of avian erythroblastosis viruses (AEVs) are generated by recombination of cellular sequences encoding the transmembrane and cytoplasmic domains of the EGFR into a viral genome. As compared to the EGFR, the erbB proteins of AEV strains R (ES4) and H are truncated at both ends, lacking most of the N-terminal EGF-binding domain as well as some C-terminal amino acids (5–8). The erbB proteins expressed by erythroblastosis-inducing proviral insertions into the EGFR gene of chickens are truncated only at their N terminus (5). Thus, N-terminal truncation is a consistent correlate of the induction of erythroblastosis by the EGFR whereas C-terminal truncation is not.

We have reported the frequent occurrence of new *erbB*-transducing viruses in cases of avian leukosis virus-induced erythroblastosis (9). During the early passage of these viruses, we realized that some erythroblastosis-inducing transductions are accompanied by angiosarcoma-inducing transductions of c-*erbB* (10). We also observed that these recently isolated erythroblastosis-inducing viruses, unlike two prior isolates of erythroblastosis-inducing viruses (AEV-R and AEV-H), were unable to transform fibroblasts or cause fibrosarcoma (9, 10). These viruses also differed from AEV-R in that their ability to induce erythroblastosis was restricted to inbred line 15_1 chickens or crosses of 15_1 chickens (11). In this manuscript we have used 11 of these recently isolated viruses as well as AEV-R and AEV-H to show that differences in the transforming potential of *erbB* viruses correlate with differences in *erbB* sequences encoding the C-terminal domain of the EGFR.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Virus Stocks. Virus stocks were prepared from filtered 10% (wt/vol) tumor homogenates and by cocultivating chicken embryo fibroblasts with tumor tissue (10). Infectious AEV-H was recovered by cotransfection of cloned AEV-H DNA (12) and cloned Rous-associated virus (RAV)-1, DNA into turkey fibroblasts as described (13). A stock of AEV-R (ES4) with RAV-1 helper virus was obtained from T. Graf.

Oncogenicity Tests. Disease potential was tested by intravenous and wing-web inoculation of 0.2 ml of virus stocks into 1-week-old crosses of 15_1 with K28 chickens or into K28 chickens. The development of erythroblastosis was monitored by examining blood smears at biweekly intervals. Wing webs were observed for solid tumors by palpation. Chickens that exhibited large numbers of immature erythroid cells in their peripheral blood or general lethargy were sacrificed and autopsied. The spleens, livers, kidneys, thymus, and lungs, as well as any hemorrhagic lesions or nodules, were subjected to histologic analyses. Inoculated birds were observed 8 weeks for disease.

Focus Assays. Fibroblasts from K28 or line 15_1 chicken embryos were used in focus assays (10).

DNA Analyses. Isolation of DNA from tumors or infected cells, restriction enzyme digestions, agarose gel electrophoresis, transfer to nitrocellulose filters, hybridization with nick-translated DNAs, post-hybridization washing, and autoradiography were as described (9).

Viral RNA Blots. RNA in the particulate fraction of virus stocks (prepared as for reverse-transcriptase assay, ref. 14) was blotted onto nitrocellulose as described by White and Bancroft (15). Subsequent processing of the filters was performed as for DNA blots (9).

RESULTS

Isolation of *erbB*-Transducing Viruses with Different Disease Potentials. Fifty percent of avian leukosis virus-induced erythroleukemias in (15_1 × K28) × K28 chickens contain newly generated *erbB*-transducing viruses (9). From seven

Abbreviations: EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; AEV, avian erythroblastosis virus; AAV, avian angiosarcoma-inducing virus; AFV, avian fibrosarcoma-inducing virus; bp, base pair(s); kb, kilobase(s); RAV, Rous-associated virus.

such erythroleukemias we have obtained 11 virus isolates (Table 1). Stocks of each of the virus isolates were obtained by passaging viruses in chickens and then in cultured cells. During these passages, viruses were followed by analyzing DNA from tumors or cultures for characteristic *erbB*-containing *Eco*RI fragments of proviral DNA (9). Data are reported for stocks that exhibited a consistent disease potential associated with a single and consistently sized proviral *erbB* fragment. During the early passage of some of the tumor homogenates, induced tumors contained proviruses that had not been detected in the original tumor. Some but not all of these *erbB*-containing viruses were detected in subsequent passages. Thus, the viruses reported here represent a subset of the viruses that appeared in the tumors that exhibited transductions. This subset of viruses causes tumors and undergoes efficient transmission.

As expected, each erythroleukemic chicken yielded virus that causes erythroblastosis. These viruses were named AEVs and designated by the number of the chicken in which they originated. The erythroblastosis induced by these viruses was like that of *erbA* deletion mutants of AEV-R in that leukemic cells consisted of a mixture of erythroblasts and polychromatic erythrocytes (16, 17). However, unlike AEV-R (or *erbA* deletion mutants of AEV-R), none of the seven AEV isolates caused fibrosarcoma (9, 10).

Two chickens, 4890 and 5005, also yielded viruses that cause angiosarcoma while a third, 5014, yielded both a virus that causes angiosarcoma and a virus that causes fibrosarcoma (Table 1). The angiosarcoma-inducing viruses were named AAVs, and the fibrosarcoma-inducing virus was named AFV. Chickens with angiosarcomas had many hemorrhagic lesions in their internal organs as well as in their skin and muscle. These lesions contained cells with endothelial, mesenchymal, and fibroblastic appearance (10). The angiosarcomas induced by different viruses had slightly different characteristics—angiosarcomas induced by AAV-4890 contained more cells of endothelial appearance than the lesions induced by AAV-5005 (10) or AAV-5014. Chickens inoculated with AFV-5014 developed widely disseminated fibrosarcomas. These fibrosarcomas consisted almost exclusively of spindle-shaped cells with only occasional centers of possible endothelial involvement. The type of disease induced by each of the new viruses was not influenced by the route of inoculation.

Tests for the Disease Potential of AEV-R and AEV-H. Since the disease potentials of the virus isolates were different from those reported for AEV-R and AEV-H (12, 18, 19), we obtained AEV-R and AEV-H and tested them for disease potential under our conditions. All of the *erbB*-transducing viruses as well as AEV-R and AEV-H are defective viruses that rely on proteins supplied by helper viruses for their replication. To eliminate possible contributions of helper viruses to differences in disease potential, stocks of AEV-R and AEV-H were prepared using the RAV-1 helper found in the stocks of the virus isolates. These stocks were then tested for disease potential following intravenous as well as wing-web inoculation into 15_1-related chickens.

Stocks of AEV-R exhibited a stable disease potential during passage whereas stocks of AEV-H exhibited a changing disease potential with passage (see also ref. 19). Also, different disease potentials were observed for three stocks of AEV-H that had been independently recovered from molecularly cloned AEV-H DNA. To obtain a stock that could be tested for the true disease potential of AEV-H, a stock of AEV-H that caused erythroblastosis and angiosarcoma was subjected to end-point dilution in chickens. Chickens with cases of erythroblastosis that occurred near or at the end point of the dilution series were then used to prepare stocks (filtered homogenates of bone marrow). These stocks had titers of from 1×10^3 to 1×10^4 infectious units of AEV-H per ml.

Stocks of AEV-R as well as stocks of AEV-H (obtained as described above) caused erythroblastosis following intravenous inoculation and erythroblastosis as well as fibrosarcoma following wing-web inoculations. The fibrosarcomas were unlike the fibrosarcoma induced by AFV-5014, in that they did not occur following intravenous inoculations and tended not to occur in the internal organs of chickens following wing-web inoculations. Proviral *erbB* sequences in cases of AEV-H-induced erythroblastosis and fibrosarcoma had restriction endonuclease maps that were indistinguishable from those observed for the molecularly cloned DNA of AEV-H.

Host Pedigree Restriction of Disease Induction. The virus isolates as well as AEV-R and AEV-H were tested for disease induction in crosses of inbred line 15_1 with noninbred K28 chickens and in K28 chickens. These tests were of interest because inbred line 15_1 chickens carry a dominant trait for high susceptibility to *erbB*-induced erythroblastosis (11). In the tests for pedigree restriction, AEVs, AAVs, and AFV were inoculated intravenously into chickens. The AEVs (four were tested) caused disease in 15_1-related but not in K28 chickens (Table 2). In contrast, AEV-R and AEV-H caused erythroblastosis in K28 as well as crosses of 15_1 and K28

Table 1. Passage and disease potential of seven *erbB* transducing viruses

Originating chicken	Characteristic *Eco*RI Fragment*	Disease	Designation
4920	2.1	Erythroblastosis	AEV-4920
4883	2.2	Erythroblastosis	AEV-4883
5009	3.0	Erythroblastosis	AEV-5009
4890	3.5	Erythroblastosis	AEV-4890
	3.3	Angiosarcoma	AAV-4890
5040	4.2	Erythroblastosis	AEV-5040
5005	4.3	Erythroblastosis	AEV-5005
	4.1	Angiosarcoma	AAV-5005
5014	2.1	Erythroblastosis	AEV-5014
	3.5	Angiosarcoma	AAV-5014
	4.2	Fibrosarcoma	AFV-5014

*Size in kilobases (kb) of the proviral *Eco*RI fragment that hybridizes with the BAM and BAM-RI probes (see Fig. 1*A*).

Table 2. Fibroblast transformation and host restriction of oncogenicity of *erbB*-transducing viruses

Virus*	Host susceptibility: Inoculum, *erbB* units†	Host susceptibility: No. diseased/no. tested: 15_1-related	Host susceptibility: No. diseased/no. tested: K28	Focus formation, foci per *erbB* unit
AEV-5005	2×10^5	10/10‡	0/10	<10
AEV-5009	2×10^5	5/5‡	0/6	<10
AEV-5040	4×10^3	5/7§	0/10	<10
AEV-5014	4×10^4	4/6§	0/10	<10
AEV-H¶	4×10^2	NT	3/3	$>10^4$‖
AEV-R	5×10^4	NT	7/7	$>10^4$
AAV-5005	1×10^3	NT	3/3	$>10^4$‖
AAV-4890	5×10^4	NT	3/3	$>10^4$
AFV-5014	1×10^4	NT	6/7	$>10^4$
AAV-5014	1×10^5	8/8§	NT	$>10^4$

NT, not tested.
*The helper virus in each stock was RAV-1.
†*erbB* units were calculated from densitometric tracings of autoradiograms of slot blots of virion RNA hybridized with ^{32}P-labeled BAM-RI probe (see Fig. 1). Densities were normalized using a stock of AEV-R of known focus-forming titer as standard.
‡Line 15_1 × K28 chickens.
§(15_1 × K28) × (15_1 × K28) or (15_1 × K28) × K28 chickens.
¶The stock of AEV-H was prepared from the bone marrow of a chicken inoculated with an end-point dilution of AEV-H.
‖Cells in foci were fibroblastic in appearance.

chickens. The three AAVs and AFV-5014 also were not pedigree restricted in their disease potential.

To quantitate the level of this host pedigree restriction, estimates of the titer of *erbB* sequences in each virus stock were obtained from densitometer tracings of slot blots of virion RNA hybridized with the ^{32}P-labeled BAM-RI probe (see Fig. 1). The amounts of *erbB*-related RNA in each stock were normalized to those present in a standard stock of AEV-R. The results of these tests indicated that the pedigree restriction of the AEV isolates was marked with inocula more than 500 times that required for AEV-H to induce erythroblastosis in K28 chickens failing to cause disease in K28 (Table 2).

Fibroblast Transformation. The AEVs, AAVs, and AFV as well as AEV-R and AEV-H were tested for their ability to transform chicken embryo fibroblasts, as judged by focus formation on monolayers grown under agar (Table 2). In these tests, focus-forming units were compared with the amount of *erbB*-related RNA in each virus stock. The new AEVs were less efficient by a factor of 1000 than AEV-R in fibroblast transformation. This was true even when fibroblasts derived from line 15_1 chickens were used for the focus assays. In contrast, the three AAVs, AFV-5014, and AEV-H had ratios of focus-forming units to *erbB* units comparable to that of AEV-R. The appearance of foci varied with the different viruses, with cells transformed by AEV-H and AAV-5005 being more fibroblastic in appearance than cells transformed by AAV-4890, AAV-5014, AFV-5014, and AEV-R.

Transduced *erbB* Genes. The AEVs, AAVs, and AFV-5014 were analyzed for proviral *erbB* sequences using Southern blots of restriction enzyme-digested tumor DNAs. Each of the viruses hybridized with probes derived from the 5′, central (*src*-related), and 3′ regions of the v-*erbB* of AEV-R. None of the viruses hybridized with a probe containing sequences encoding the EGF-binding domain of the human EGFR (3) even though this probe easily detected a discrete sequence in chicken genomic DNA. Thus, like AEV-R and AEV-H, the transductions appear to encode an N-terminal truncated form of the EGF receptor.

Proviral DNAs of the viruses were compared for the sizes of restriction endonuclease fragments containing sequences encoding the transmembrane, kinase, and C-terminal domains of the EGFR. Since independent transductions have different points of recombination between viral and *erbB* sequences (9), v-*erbB* sequences encoding the transmembrane domain of the EGFR were compared in viruses that originated in the same chicken but had different disease potentials. No size differences could be detected in the 5′-junction fragments of viral and *erbB* sequences in molecularly cloned DNAs of AEV-5005 and AAV-5005 or in DNAs from tumors induced by AEV-4890 and AAV-4890. Thus the differences in disease potential of these pairs of viruses did not correlate with obvious differences in 5′-*erbB* sequences. Sequences encoding the kinase domain of the EGFR also

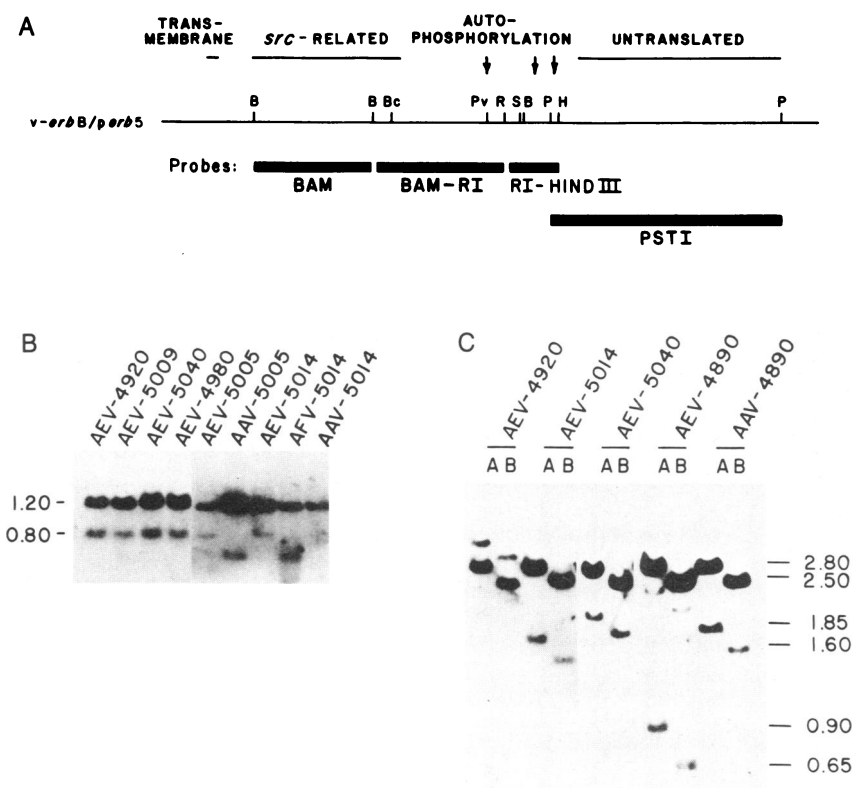

FIG. 1. Analysis of transduced *erbB* genes in proviral DNAs. (*A*) Composite map based on published sequences of AEV-H *erbB* (6) and *perb5*, a cDNA clone derived from an avian leukosis virus-induced erythroleukemia (5). Locations of sequences specifying the transmembrane and *src*-related domains, the major autophosphorylation sites of the *erbB*/EGFR protein, and the 3′-untranslated region of the *erbB*/EGFR mRNA are indicated. The *Hin*dIII recognition site overlaps with the penultimate codon at the C terminus. The 3′ *Pst* I site was generated during cDNA cloning (5). The indicated restriction sites and probes were used in studies depicted in *B* and *C* and others described in the text. B, *Bam*HI; Bc, *Bcl* I; Pv, *Pvu* II; R, *Eco*RI; P, *Pst* I; H, *Hin*dIII. (*B*) Analysis of the 3′-coding sequences of the v-*erbB*s. Shown are representative autoradiograms of Southern blots of DNAs digested with *Bcl* I and *Hin*dIII and hybridized with ^{32}P-labeled RI-HINDIII probe. Estimated fragment sizes are given in kb. The 1.2-kb fragment is the only cellular fragment detected by this probe; the smaller fragments are of proviral origin. (*C*) Analysis of *erbB* coding and noncoding sequences in *erbB*/RAV-1 3′-junction fragments. Shown are representative autoradiograms of Southern blots of DNAs digested with *Eco*RI (lanes A) or both *Eco*RI and *Hin*dIII (lanes B) and hybridized with the ^{32}P-labeled PST probe. Fragment sizes are given in kb. The 2.8- and 2.5-kb bands are the only cellular fragments detected by this probe; all other fragments are of proviral origin.

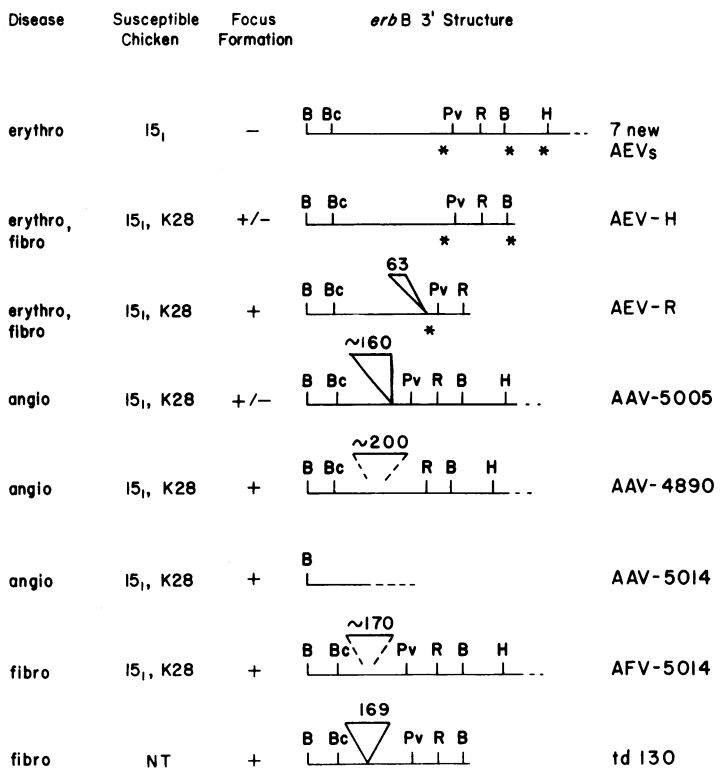

FIG. 2. Summary of phenotypic properties and 3′ structures of 14 transduced *erbB* genes. Each line shows summarized results of oncogenicity and cell transformation tests and a schematic diagram of *erbB* gene structures on the 3′ side of the *Bam*HI site located at the 3′ end of the *src*-related region of *erbB*. Asterisks indicate the location of sequences encoding the homologs of the major sites of autophosphorylation of the human EGFR (3). Dashed horizontal lines indicate areas where the extent of *erbB* homology is uncertain. Sizes of deletions are given in bp with horizontal lines in scale. The locations of deletions are indicated by solid lines if their boundaries are known or by dashed lines, if they are only mapped to a region. Gene structure data for AEV-H and td130 are from Yamamoto *et al.* (6) and data for AEV-R are from J. M. Bishop (personal communication).

appeared to be similar in viruses with different disease potentials. This was shown by all of the viruses having ≈560-base pair (bp) *src*-related *Bam*HI fragments. These fragments comigrated with those found in AEV-R and AEV-H.

The *erbB* genes were next tested for sequences encoding the C-terminal domain of the EGFR using a number of different restriction endonuclease digests of tumor DNAs as well as molecularly cloned DNAs from AEV-R, AEV-H, AEV-5005, and AAV-5005. Particularly informative were *Bcl* I plus *Hin*dIII double digestions in which each of the AEVs displayed a discrete 0.8-kb fragment that hybridized with the RI–HINDIII probe (Fig. 1*B*). The sequence of a cDNA derived from a provirally promoted c-erbB mRNA contains a single *Bcl* I site and a single *Hin*dIII site, separated by 798 bp (ref. 5, see Fig. 1*A*). The *Hin*dIII site overlaps with the penultimate codon of the chicken EGFR (5). Thus each of the viruses appear to encode C termini that are complete up to the penultimate amino acid. Evidence that sequences in the AEVs encode the complete C terminus of the EGFR is presented in Fig. 1*C*. In these analyses, the *Eco*RI fragment containing the 3′ junction of EGFR and viral sequences was digested with *Hin*dIII. *Hin*dIII digestion reduced the apparent sizes of the junction fragments by ≈250 bp as would be predicted from the presence of a *Hin*dIII site 248 bp to the 3′ side of the *Eco*RI site in the chicken EGFR cDNA (Fig. 1*A*). The *Hin*dIII–*Eco*RI fragments containing the 3′ junction of *erbB* and viral sequences hybridized with the PSTI probe (see lanes B in Fig. 1*C*) indicating the presence of EGFR sequences on the 3′ side of the *Hin*dIII site. Moreover, the proviral *erbB* genes in each of these erythroblastosis viruses had restriction endonuclease maps that were indistinguishable from those observed for the molecularly cloned c-erbB cDNA (Fig. 2). These analyses indicate that each of the seven AEVs contains sequences encoding the complete C-terminal domain of the EGFR. Thus the newly isolated erythroblastosis viruses are different from AEV-H and AEV-R, which are truncated for sequences encoding the C terminus of the EGFR (5, 6, 8).

In contrast to the AEVs, the angiosarcoma and fibrosarcoma-inducing viruses had internal deletions or substantial truncations in sequences encoding the C-terminal domain of the EGFR. The *Bcl* I-*Hin*dIII *erbB* fragments detected in proviral DNA of AAV-5005, AAV-4890, and AFV-5014 were shorter than the 0.8-kb fragments observed in the AEVs (see digests for AAV-5005 and AFV-5014 in Fig. 1*B*). Analyses of the 3′-junction fragments in these viruses using *Eco*RI and *Eco*RI plus *Hin*dIII digestions indicated that these shorter fragments represented internal deletions in EGFR sequences (see AAV-4890 in Fig. 1*C*). The deletions in AAV-5005, AFV-5014, and AAV-4890 were mapped to the same general region of *erbB* as the 169-bp internal deletion previously reported in td130, a derivative of AEV-H that induces only fibrosarcoma (6, 12). The results of this mapping are summarized in Fig. 2.

The *erbB* gene of AAV-5014 was found to have a substantial truncation in sequences encoding the C-terminal domain of the EGFR. The proviral DNA of AAV-5014 hybridized with the BAM-RI probe but had no homology to the RI-HINDIII or PST probes for EGFR sequences. The extent of the truncation was studied by testing for the presence of *Pvu* II, *Eco*RI, *Sac* I, *Bam*HI, and *Hin*dIII restriction endonuclease sites in the v-*erbB* gene of AAV-5014. These analyses indicated that the *erbB* gene in AAV-5014 did not contain sites including and to the 3′ side of the *Pvu* II site in the EGFR c-DNA.

DISCUSSION

Different Transforming Potentials Correlate with Differences in 3′ v-*erbB* Sequences. The 14 *erbB*-transducing viruses summarized in Table 2 exhibit the following 4 different disease potentials: (*i*) the ability to cause erythroblastosis in line 15_1 chickens coupled with the inability to transform fibroblasts or cause disease in random bred chickens, (*ii*) the ability to cause erythroblastosis and fibrosarcoma in any chicken, (*iii*) the ability to cause angiosarcoma in any chicken, and (*iv*) the ability to cause fibrosarcoma in any chicken. Each of these disease potentials correlated with differences in sequences encoding the C-terminal domain of the EGFR.

Seven viruses were able to cause erythroblastosis in line 15_1 chickens but were unable to transform fibroblasts or

cause erythroblastosis in random bred chickens. The v-*erbB* genes of each of these viruses contained sequences encoding the complete C terminus of the EGFR. Thus viruses that express v-*erbB* genes encoding the complete C terminus of the EGFR have very limited transforming potential.

Two viruses, AEV-R and AEV-H, were able to cause erythroblastosis in noninbred chickens and could transform fibroblasts and cause fibrosarcoma following wing-web (but not intravenous) inoculation. Both of these viruses encode C-terminally truncated erbB proteins that lack the homolog of at least one of the sites of EGF-stimulated autophosphorylation of the human EGFR (4–6, 8). This is a provocative result since it suggests that loss of sites for autophosphorylation increases the transforming potential of erbB proteins for erythroblasts and for fibroblasts. The result also suggests that the high susceptibility of the erythroid cells of line 15_1 chickens to *erbB*-induced transformation (11) is determined by an atypical response to autophosphorylation.

The two remaining transforming potentials are represented by three angiosarcoma-inducing viruses and one fibrosarcoma-inducing virus. Apart from causing different diseases, these viruses were similar in being able to transform fibroblasts, being able to cause disease in any chicken and having substantial perturbations in v-*erbB* sequences encoding the C terminus of the EGFR. Two of the angiosarcoma viruses as well as the fibrosarcoma virus had large internal deletions whereas the third angiosarcoma virus had a substantial truncation in sequences encoding the C terminus. These results complement the finding of an internal deletion in td130, a derivative of AEV-H that only induces fibrosarcomas (6). The internal deletion in td130 results in truncation of the 222 C-terminal amino acids of the chicken EGFR.

Most *erbB*-Transducing Viruses Cause Disease in Only One Cell Lineage. Twelve out of the 14 *erbB*-transducing viruses in Fig. 2 cause a single disease. This remarkable specificity in pathogenic potential does not appear to be the result of differences in tissue tropisms since all of the *erbB*-transducing viruses in our tests were grown in the presence of the same helper virus. Also, the differences in disease specificity are not easily attributed to differences in the time of onset of erythroblastosis, angiosarcoma, and fibrosarcoma. With the exception of AEV-R and AEV-H, where the induction of fibrosarcoma takes longer than the induction of erythroblastosis (20), the times of onset of the different *erbB*-induced diseases are remarkably similar. Chickens infected with stocks containing a mixture of viruses reproducibly develop diseases caused by each of the viruses in the mixture. The specificity in disease induction by viruses encoding erbB proteins with different C termini implies that the tissues that are targets for transformation by these viruses are differentiated with respect to their response to the EGFR. The erbB proteins in the viruses we have described should help resolve the function and tissue-specific interactions of the C-terminal domain of the EGFR.

We would like to thank Dr. B. A. Woda, Department of Pathology, University of Massachusetts Medical School, for discussion of histologic diagnoses. We are indebted to B. P. Blais for expert and invaluable help with pathogenicity tests. We thank S. Kawai for providing cloned AEV-H DNA, H.-J. Kung for cloned p*erb5* DNA, T. Graf for help in the production of AEV-R(RAV-1), and J. M. Bishop for permission to cite unpublished sequence data for AEV-R. This work was supported by Public Health Service Postdoctoral Fellowship 1 F32 CA 07793-01 to D.C.G., Research Grants R01 CA27223 and R01 CA23086 to H.L.R., and Core Grant P30 CA12708 to the Worcester Foundation for Experimental Biology.

1. Downward, J., Yarden, Y., Mayes, E. L. V., Scrace, G., Totty, N., Stockwell, P., Ullrich, A., Schlessinger, J. & Waterfield, M. D. (1984) *Nature (London)* **307**, 521–527.
2. Ullrich, A., Coussens, L., Hayflick, J. S., Dull, T. J., Gray, A., Tam, A. W., Lee, J., Yarden, Y., Libermann, T. A., Schlessinger, J., Downward, J., Mayes, E. L. V., Whittle, N., Waterfield, M. D. & Seeburg, P. H. (1984) *Nature (London)* **309**, 418–425.
3. Hunter, T. (1984) *Nature (London)* **311**, 414–416.
4. Downward, J., Parker, P. & Waterfield, M. D. (1984) *Nature (London)* **311**, 483–485.
5. Nilsen, T. W., Maroney, P. A., Goodwin, R. G., Rottman, F. M., Crittenden, L. B., Raines, M. A. & Kung, H.-J. (1985) *Cell* **41**, 719–726.
6. Yamamoto, T., Nishida, T., Miyajima, N., Kawai, S., Ooi, T. & Toyoshima, K. (1983) *Cell* **35**, 71–78.
7. Debuire, B., Henry, C., Benaissa, M., Claverie, J. M., Saule, S., Martin, P. & Stehelin, D. (1984) *Science* **224**, 1456–1459.
8. Nishida, T., Sakamoto, S., Yamamoto, T., Hayman, M., Kawai, S. & Toyoshima, K. (1984) *Gann* **75**, 325–333.
9. Miles, B. D. & Robinson, H. L. (1985) *J. Virol.* **54**, 295–303.
10. Tracy, S. E., Woda, B. A. & Robinson, H. L. (1985) *J. Virol.* **54**, 304–310.
11. Robinson, H. L., Miles, B. D., Catalano, D. E., Briles, W. E. & Crittenden, L. B. (1985) *J. Virol.* **55**, 617–622.
12. Yamamoto, T., Hihara, H., Nishida, T., Kawai, S. & Toyoshima, K. (1983) *Cell* **34**, 225–232.
13. Robinson, H. L., Jensen, L. & Coffin, J. M. (1985) *J. Virol.* **55**, 752–759.
14. Robinson, H. L. (1976) *J. Virol.* **18**, 856–866.
15. White, B. A. & Bancroft, F. C. (1982) *J. Biol. Chem.* **257**, 8569–8572.
16. Frykberg, L., Palmieri, S., Beug, H., Graf, T., Hayman, M. & Vennstrom, B. (1983) *Cell* **32**, 227–238.
17. Sealy, L., Privalsky, M. L., Moscovici, G., Moscovici, C. & Bishop, J. M. (1983) *Virology* **130**, 155–178.
18. Graf, T. & Beug, H. (1978) *Biochim. Biophys. Acta* **516**, 265–299.
19. Hihara, H., Yamamoto, H., Shimohira, H., Arai, K. & Shimuzu, T. (1983) *J. Natl. Cancer Inst.* **70**, 891–894.
20. Graf, T., Fink, D., Beug, H. & Royer-Pokera, B. (1977) *Cancer Res.* **37**, 59–63.