JOURNAL OF VIROLOGY, Sept. 1980, p. 888-894
0022-538X/80/09-0888/07$02.00/0

Vol. 35, No. 3

Chromosomal Localization of *ev-1*, a Frequently Occurring Endogenous Retrovirus Locus in White Leghorn Chickens, by In Situ Hybridization

ALLAN TEREBA[1]* AND SUSAN M. ASTRIN[2]

St. Jude Children's Research Hospital, Memphis, Tennessee 38101,[1] and The Institute for Cancer Research, The Fox Chase Cancer Institute, Philadelphia, Pennsylvania 19111[2]

The distribution of 12 endogenous retrovirus loci from 13 White Leghorn flocks of diverse geographical origin was examined, using a total of 508 birds. Only one locus, designated *ev-1*, was observed to be almost universally present (506 of 508 birds) and thus predominantly homozygous in this breed of chicken. By contrast, 11 other viral loci were observed at much lower frequencies, 3 to 30%, and were occasionally restricted to individual flocks. To further characterize the commonly occurring *ev-1* viral locus and to provide a foundation for determining the mechanism by which these 12 endogenous retrovirus loci were generated, a new technique of indirect in situ hybridization was used to localize the chromosomal position of *ev-1*. By using a combination of specific probes, the position of *ev-1* was localized to a small region on the long arm of chromosome 1. This research represents one of the first instances in which a defined segment of nonreiterated DNA coding for a known set of genes has been localized to a specific region on a vertebrate chromosome by in situ hybridization.

Nucleic acid hybridization experiments have demonstrated the presence of endogenous retrovirus genomes integrated into the DNA of a wide variety of organisms (1, 22). These endogenous viral genomes do not appear to play a major role in ontogeny (5) or oncogenesis. However, their similarity to oncogenic retroviruses (11, 21, 24, 30, 34) and their ability to recombine with them (15, 32), as well as their potential for acquiring cell genes (18, 35, 36), have focused attention on their origin, mode of transcriptional regulation, and interaction with nearby host genes.

In the class of *Aves*, the presence of these endogenous retroviruses has been most extensively studied in the White Leghorn chicken. Nucleic acid hybridization has revealed without exception the presence of a small number of endogenous viral genomes related to Rous sarcoma virus (RSV) and an associated leukosis helper virus (11, 21, 24, 30, 34). Recently, these data were extended to show the presence of up to 10 distinct viral loci when restriction endonuclease-digested chicken DNA was examined for retrovirus-specific sequences (4, 9). The genetic uniqueness of several of these loci has been confirmed by animal genetic studies (4-8, 23). In this original study of over 150 birds, only one viral locus, designated endogenous virus 1 (*ev-1*), was present in all birds.

To determine the mechanisms involved in the generation of these genetically distinct endogenous retrovirus loci, we have embarked on an extensive program to define the genetic distribution of each locus within several flocks of chickens and to determine their chromosomal location. This information should help determine whether the loci have been acquired by random or specific integration of exogenous retroviruses. As a first step, we report here the distribution of 12 distinct viral loci in 13 White Leghorn flocks and describe the localization of the commonly occurring *ev-1* locus to a small region on chromosome 1 by using our sensitive technique of indirect in situ hybridization (29).

MATERIALS AND METHODS

Cells and cell DNA. Primary fibroblast cultures were prepared from 11-day-old White Leghorn embryos (SPAFAS, Norwich, Conn.; Heisdorf and Nelson, Redmond, Wash.; Regional Poultry Research Laboratory, East Lansing, Mich.; H. Robinson, Worcester, Mass.) as described by Rubin (25). DNA was prepared from cultured cells or from erythrocytes of adult birds, using phenol, as described by Varmus et al. (33).

Characterization of DNA. The digestion of cell DNA with restriction endonuclease *Sst*I (Bethesda Research Laboratories, Rockville, Md.), *Bam*HI, *Eco*RI, and *Hin*dIII (New England BioLabs, Beverly, Mass.) has been described by Astrin (4). After digestion, the DNA fragments were electrophoresed in 1% agarose gels (19) and transferred to nitrocellulose filters (Millipore HAWP00010) by the method of Southern (27) as modified by Ketner and Kelly (19). Hybridization of the transferred DNA fragments with

^{32}P-labeled Rous-associated virus type 2 (RAV-2) RNA and subsequent autoradiography have been described (4).

Metaphase chromosome preparation. For chromosome preparation, 10^6 chicken embryo fibroblasts in a volume of 1 ml of Ham's F-10 supplemented with 10% tryptose phosphate and 5% calf serum were seeded on each of several microscope slides placed in 100-mm culture dishes. Three hours later, the slides were flooded with 7 ml of Ham's F-10 supplemented with 15% calf serum and 10% tryptose phosphate (GM). Twenty hours later, medium was removed and 7 ml of GM containing 1 μM colcemid was added. After 4 h the slides were placed in 0.075 M KCl. The cells were then fixed in methanol-acetic acid (3:1, vol/vol) and squashed, using cover slips as described by Tereba et al. (29). After drying on a hot plate, slides containing 50 to 300 well-defined metaphase spreads were selected for in situ hybridization experiments using phase-contrast microscopy.

In situ hybridization. The preparation and characterization of hybridization probes containing RNA from the Prague strain of RSV, subgroup C (PR-RSV-C), or a sarcoma-defective isolate of Bratislava 77 (tdB77) attached to ^{125}I-labeled high-molecular-weight sea urchin DNA has been fully described (29). In situ hybridization reactions were performed at 39°C in 50-μl volumes that contained 40% formamide, 0.6 M NaCl, 0.06 M sodium citrate (4× SSC), and 1 to 2 ng of probe. The reactions were stopped after 16 h, washed, and prepared for autoradiography as described by Tereba et al. (29). Autoradiograms were exposed for 1 month.

Analysis of grain distribution. The background level of grains was determined by two methods. Both methods assumed the background grains were randomly distributed and, therefore, the number of background grains over each chromosome would correlate directly with the size and number of each chromosome observed. In the first method, the number of grains in regions adjacent to chromosome spreads and equivalent in area to these spreads was determined and then divided among the chromosomes on the basis of chromosome size and the number of each chromosome observed. This method produced satisfactory results in most cases, but occasionally gave excessively high or low backgrounds due to clustering of background grains. Alternatively, the background level was estimated by determining the grain density over the metaphase chromosomes containing no detectable virus-related loci. As a first approximation of the background level over each chromosome, the total number of grains over the metaphase chromosomes was divided among the chromosome groups as described in the first method. These estimated background values were then subtracted from the number of grains experimentally observed over each chromosome. Chromosomes containing excessive grains above this estimated background based on a Poisson distribution ($P < 0.01$) were assumed to contain grains due to specific hybridization and thus were eliminated from the background calculation. The remaining grains provided an experimental value of background grains over a specified area. Thus, this grain density provided the means to calculate the background over each chromosome based on chromosome size and the number of each chromosome observed. In all cases, this method agreed very well with the observed experimental data, assuming a simple Poisson distribution. In conjunction with the restriction endonuclease analysis, both methods of analyzing the in situ hybridization data resulted in the same conclusions being drawn. However, due to the more consistent results obtained by the second method, this procedure was used to calculate all background levels reported here.

RESULTS

Distribution of the *ev-1* endogenous viral locus. In our recent study using restriction endonuclease-digested DNA from over 150 uninfected White Leghorn chickens, several distinct patterns of virus-specific DNA were observed (4). Using the supposition that restriction endonuclease-digested host DNA would have a distinct and characteristic set of retrovirus-specific DNA fragments for each viral locus that was present, at least seven distinct loci were identified. Of particular interest was the universal presence of a locus designated endogenous virus-1 (*ev-1*). To obtain a broader perspective on the distribution of the *ev-1* locus in White Leghorn chickens, we examined a total of 508 birds from 13 distinct flocks. The simplest pattern of virus-specific fragments to emerge from our study was that generated from the DNA of birds containing only *ev-1*. Of the 508 birds examined, 14% contained only this locus. Restriction endonuclease patterns for *ev-1* have been published (4, 16, 20, 26). Also, *ev-1* and the flanking cellular sequences have been cloned, and a detailed map of restriction endonuclease cleavage sites has been generated by Skalka et al. (20, 26). In addition to the *ev-1* locus, 11 other loci were detected in our present study. A list of the characteristic DNA fragments for all of these observed loci, using the restriction endonuclease *Sst*I or *Bam*HI, is presented in Table 1.

The distribution of the *ev-1* locus among 508 birds from 13 flocks is presented in Table 2. For comparison, the distributions of the 11 additional loci detected in this study are also included. Loci designated *ev-2* through *-12* were present to varying degrees, ranging from 3 to 30%. In addition, several of these loci were restricted to a particular line of chicken. By contrast, *ev-1* was observed in 506 of 508 birds, even though the flocks were from different geographical areas and originated from different commercial sources. Thus it appears that *ev-1* is largely in a homozygous state. This also suggests a possible role of *ev-1* in the origination of at least some of the other endogenous retrovirus loci in White Leghorn chickens.

Chromosomal localization of the *ev-1* lo-

cus. Although the analysis of retrovirus-specific DNA fragments obtained by restriction endonuclease digestion can determine the presence and distribution of specific viral loci, the chromosomal position of these sequences and their relationship to other virus and cell genes is not defined. To determine the chromosomal position for the *ev-1* locus, we used the new technique of indirect in situ hybridization developed by Tereba et al. (29). A probe containing RNA from tdB77 attached to ^{125}I-labeled DNA was hybridized with metaphase chromosomes from embryo 5, a representative bird containing only *ev-1*.

TABLE 1. *Characteristic virus-specific restriction endonuclease fragments in White Leghorn chickens*

Locus	Mol wt of major *Sst*I fragment ($\times 10^{-6}$)	Mol wt of characteristic *Bam*HI fragment ($\times 10^{-6}$)	Reference[a]
1	5.8	3.2	(5)
2	3.7	5.1	(7)
3	3.9	4.5	(7)
4	5.4	4.5	(8)
5	12	7.8	
6	13	2.7	S. M. Astrin and H. L. Robinson, in prepn; L. B. Crittenden and S. M. Astrin, in prepn
7	8	4.7	(23)
8	11	14	Astrin and Robinson, in prepn
9	14	7	Astrin and Robinson, in prepn
10	13	8.5	Crittenden and Astrin, in prepn
11	8.0	NI[b]	
12	5.0	NI	Astrin, unpublished data; Smith and Crittenden, unpublished data

[a] These references document the segregation of each fragment pattern as a distinct genetic locus.
[b] NI, Not identified.

A representative autoradiogram of a metaphase chromosome spread hybridized with a virus-specific probe is presented in Fig. 1. This spread illustrates the ease of identifying the six large macrochromosomes without special banding techniques. However, the smaller chromosomes have been grouped into three size classes because of an inability to identify consistently these small chromosomes. Although specific hybridization was evident in many spreads, a significant background required the use of statistical analysis (Table 3). After subtracting background grains, the only chromosome containing statistically significant grains was chromosome 1. This result suggests that *ev-1* is on chromosome 1 and is consistent with data obtained by chromosome fractionation (17) and in situ hybridization studies (29) using chromosomes from birds of unknown endogenous virus genotypes.

To confirm this result and to corroborate the existence of only 1 retrovirus locus in embryo 5, an in situ hybridization experiment was performed by using a probe containing RNA from PR-RSV-C attached to ^{125}I-labeled DNA (Table

TABLE 2. *Distribution of endogenous retrovirus loci in White Leghorn chickens*

Chicken flock	Total no. of birds	% of birds containing specific *ev* loci											
		ev-1	*ev-2*	*ev-3*	*ev-4*	*ev-5*	*ev-6*	*ev-7*	*ev-8*	*ev-9*	*ev-10*	*ev-11*	*ev-12*
Inbred lines													
6_3	24[a]	100	0	100	0	0	0	0	0	0	0	0	0
7_2	26[a]	100	100	0	0	0	0	0	0	0	0	0	0
15_B	60[a]	100	0	0	0	0	0	100	0	0	0	0	0
15_1	15	100	0	100	0	0	100	0	0	0	0	7	100
C	11	100	0	0	0	0	0	100[b]	0	0	100[b]	0	0
15_{14}	12	100	0	0	0	0	89	0	0	0	100	100	0
15_{15}	10	100	0	0	0	0	100	0	0	0	90	20	0
Noninbred lines													
SPAFAS line 11 *gs*$^-$ *chf*$^-$	200	99	0	0	56	25	0	0	ND[c]	0	0	0	0
SPAFAS line 11 *gs*$^+$ *chf*$^+$	10	100	0	100	30	70	20	0	0	0	0	0	0
SPAFAS line 11 *gs*$^-$ *chf*$^+$	6	100	0	0	83	0	33	0	17	67	0	0	0
Heisdorf and Nelson *gs*$^+$ *chf*$^+$	2	100	0	100	0	0	0	0	0	0	0	0	0
Heisdorf and Nelson *gs*$^-$ *chf*$^-$	3	100	0	0	33	67	0	0	0	0	0	0	0
K(−)	34[a]	100	0	0	12	12	0	0	0	0	0	0	0
K16	32[a]	100	0	100	38	0	25	0	ND	0	0	0	0
K18	24[a]	100	0	0	71	0	100	0	79	58	0	0	0
K28	39[a]	100	0	0	0	0	0	0	0	0	0	0	0
Total	508	99.6	5	16	30	12	14	14	7	4	6	3	3

[a] Birds chosen so as to completely represent the gene pool in the breeding stock of these lines.
[b] Identified by *Sac*I and *Bam*HI digestion, not confirmed by genetic analysis.
[c] ND, Not determined, additional restriction endonuclease analysis required for identification.

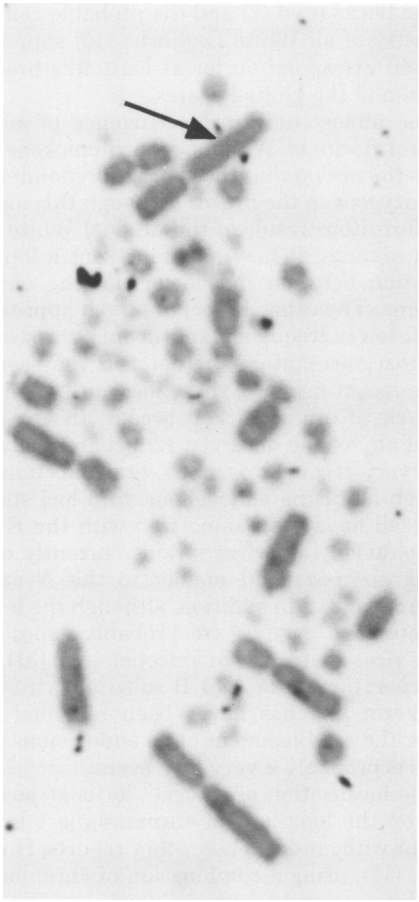

FIG. 1. *Metaphase chromosome spread hybridized with a PR-RSV-C RNA-containing probe. Metaphase chromosomes were prepared from embryo 5 which contained only ev-1 and were then hybridized with a [^{125}I]DNA probe containing PR-RSV-C RNA. Arrow represents the position of ev-1 as determined by statistical analysis.*

4). This probe would hybridize both to the *ev-1* sequence and to a normal cell gene which shares extensive homology to the viral *src* gene (28). Theoretically, the number of excess grains over each of these loci would be the same even though the sequence of the *ev-1* locus is many times larger (13). This has been shown to be the case experimentally for uncharacterized viral loci (29). Statistically significant grains above background were present only over two chromosome groups, chromosome 1, confirming the previous experiment using tdB77 RNA, and the chromosome group 10 through 12, confirming previous analyses which have localized the endogenous *src* gene to a small macrochromosome (17, 29). Furthermore, the number of excess grains over chromosome 1 and the chromosome group 10 through 12 are approximately equal (1.1:1), confirming the existence of an equal number of *ev-1* loci in relationship to the endogenous *src* gene.

Specific localization of *ev-1*. The localization of endogenous viral loci to specific chromosomes would provide some help in analyzing the origin of the viral loci. However, a more precise localization would be necessary to hypothesize any detailed mechanism and possible interaction between nearby genes. This site-specific localization has been accomplished for the *ev-1* locus by plotting the grain distribution on chromosome 1, using data obtained from the hybridization of both tdB77 and PR-RSV-C RNA-containing probes. Figure 2 clearly shows one major peak at position 0.27, indicating the location of *ev-1*. The size and shape of this peak

TABLE 3. *Grain distribution over metaphase chromosomes from embryo 5 hybridized with a tdB77 RNA-containing probe*[a]

Chromosome no.	Total no. of grains[b]	No. of grains minus background	Probability of random occurrence[c]
1	83	32	<0.0001
2	31	−6	>0.01
3	21	−6	>0.01
4	17	−2	>0.01
Z	11	2	>0.01
6	16	2	>0.01
7-9, W	35	8	>0.01
10-12	19	4	>0.01
Micro	43	−2	>0.01

[a] Only *ev-1* was detected in this embryo by restriction endonuclease analysis.
[b] From 139 metaphase chromosome spreads.
[c] Based on a Poisson distribution.

TABLE 4. *Grain distribution over metaphase chromosomes from embryo 5 hybridized with an RSV RNA-containing probe*[a]

Chromosome no.	Total no. of grains[b]	No. of grains minus background	Probability of random occurrence[c]
1	247	77	<0.0001
2	116	−5	>0.01
3	84	−3	>0.01
4	62	4	>0.01
Z	39	11	>0.01
6	29	−18	0.01
7-9,W	87	−5	>0.01
10-12	120	68	<0.0001
Micro	165	15	>0.01

[a] Only *ev-1* was detected in this embryo by restriction endonuclease analysis.
[b] From 119 metaphase chromosome spreads.
[c] Based on a Poisson distribution.

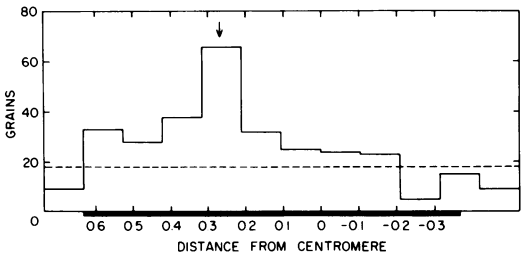

FIG. 2. *Distribution of silver grains on chromosome 1 of embryo 5 hybridized in situ with virus-specific probes. The distribution of silver grains on chromosome 1 was determined from autoradiograms of chromosomes from embryo 5 hybridized in situ with tdB77 and PR-RSV-C RNA-containing probes. The broken line indicates the estimated level of background silver grains. The length of chromosome 1 is defined as 1 unit and distance is measured from the centromere, with the long arm having positive values and the short arm having negative values. The arrow at 0.27 indicates the probable location of ev-1.*

are consistent with the emission of ^{125}I (3, 14) and demonstrate the ability of this isotope to localize genes to a relatively small area. Similar experiments using chromosomes from four other genetically distinct embryos which contain *ev-1* have all yielded a peak at this position with a mean location of 0.26 and a standard deviation of ±0.02 units (A. Tereba and S. M. Astrin, manuscript in preparation).

DISCUSSION

By analyzing the retrovirus-specific fragments from restriction endonuclease-digested chicken DNA, we have demonstrated the widespread occurrence of a specific endogenous retrovirus locus, *ev-1,* in 13 different flocks of White Leghorn chickens. This distribution is unique in that 11 other endogenous viral loci detected in this study all occurred in a minority of birds and several were restricted to a single flock. To characterize further this *ev-1* locus, its chromosomal position has been localized to a small region on the long arm of chromosome 1 by our newly developed technique of indirect in situ hybridization. This represents one of the first examples whereby a reasonably well characterized nonreiterated vertebrate gene has been localized to a specific chromosomal site by in situ hybridization.

In this study, we have restricted our observations to chickens from several White Leghorn flocks, including seven highly inbred lines. Thus, our observations may not be universally applied. In fact, our data (9) and those of others (16) suggest that other chicken breeds have many more endogenous viral loci and some may lack *ev-1.* However, the diverse geographical origin of the flocks used (2) and the probable common ancestry of all White Leghorns (10) supports a general extrapolation for at least this breed of chicken in the United States.

The almost universal occurrence of *ev-1* in several flocks of White Leghorn chickens supports the notion that this locus is predominantly homozygous in the breed. Although this may be the fortuitous result of the original White Leghorn matings, it may also represent a long association between this locus and the chicken genome. The other 11 loci observed appear at a much lower frequency and in many cases in a heterozygous state. Whether this represents a more recent origin of these loci must await the analysis of other chicken breeds. In any case, few if any of the observed 12 loci are likely due to a very recent exogenous viral infection. Although all of the endogenous viral loci studied in detail have been associated with the E subgroup, avian retrovirus strains currently circulating are restricted mainly to the A and B subgroups (31). In addition, although the female gametes and zygotes are probably exposed to high virus levels in an infected hen (31), the incorporation of an A or B subgroup virus into the germ line has never been recorded (31). Thus, the establishment of an endogenous virus locus is probably a very rare event.

The localization of the *ev-1* locus at position 0.26 on the long arm of chromosome 1 is consistent with, and extends, other reports. Hughes et al. (17), using a combination of chromosome fractionation and Southern blotting technology, reported that an endogenous virus locus was on a large macrochromosome. In addition, our previous article (29) localized three viral loci on the long arm of chromosome 1 from a White Leghorn chicken of unknown endogenous viral genotype. Our localization of the *ev-1* locus at position 0.27 here corresponds extremely well with the middle locus (0.275) in the previous study.

The localization of *ev-1* on chromosome 1 is significant in that it demonstrates the ability to localize defined nonreiterated vertebrate genes and thus provides the foundation for examining the chromosomal distribution of endogenous retrovirus loci. Our in situ hybridization approach will allow us to determine whether there is a pattern to the distribution of *ev* loci on the chromosomes. Such information will be useful in examining the possibility that some of the loci are derived by a mechanism other than germ line infection.

Finally, in a general sense, the ability to localize specific nonreiterated vertebrate genes by in situ hybridization should provide the tools necessary to understand the organization of vertebrate chromosomes. Not only would this apply

to individual organisms, but also by studying related species, evolutionary constraints and freedoms on gene location could be determined.

ACKNOWLEDGMENTS

We thank Michael Lai for providing highly purified 35S viral RNA and R. Shah, M. Grynkiewicz, D. Keefer, and J. Fang for their expert technical assistance.

This work was supported by grants PCM 78-03253 and PCM 76-09721 from the National Science Foundation; by Public Health Service Cancer Center support (CORE) grant CA 21765 from the National Cancer Institute, biomedical research support grant RR 05584 from the National Institutes of Health, and grants CA 06927 and RR 05539 from the National Institutes of Health; by an appropriation from the Commonwealth of Pennsylvania; and by ALSAC.

LITERATURE CITED

1. **Aaronson, S. A., and J. R. Stephenson.** 1976. Endogenous type-C RNA viruses of mammalian cells. Biochim. Biophys. Acta **458**:323-354.
2. **Abplanalp, H.** 1979. Inbred lines: chickens, p. 611-613. *In* P. L. Altman and D. D. Katz (ed.), Inbred and genetically defined strains of laboratory animals. Federation of American Societies for Experimental Biology, Bethesda, Md.
3. **Ada, G. L., J. H. Humphrey, B. A. Askonas, H. O. McDevitt, and G. J. V. Nossal.** 1966. Correlation of grain counts with radioactivity (^{125}I and tritium) in autoradiography. Exp. Cell Res. **41**:557-572.
4. **Astrin, S.** 1978. Endogenous viral genes of the White Leghorn chicken: common site of residence and sites associated with specific phenotypes of viral gene expression. Proc. Natl. Acad. Sci. U.S.A. **75**:5941-5945.
5. **Astrin, S. M., E. G. Buss, and W. S. Hayward.** 1979. Endogenous viral genes are nonessential in the chicken. Nature (London) **282**:339-341.
6. **Astrin, S. M., L. B. Crittenden, and E. G. Buss.** 1979. ev 3, a structural gene locus for endogenous virus, segregates with the gs$^+$ chf$^+$ phenotype in matings of line 6_3 chickens. Virology **99**:1-9.
7. **Astrin, S. M., L. B. Crittenden, and E. G. Buss.** 1980. *ev* 2, a genetic locus containing structural genes for endogenous virus, codes for Rous-associated virus type 0 produced by line 7_2 chickens. J. Virol. **33**:250-255.
8. **Astrin, S. M., and H. L. Robinson.** 1979. *Gs*, an allele of chickens for endogenous avian leukosis viral antigens, segregates with *ev* 3, a genetic locus that contains structural genes for virus. J. Virol. **31**:420-425.
9. **Astrin, S. M., H. L. Robinson, L. B. Crittenden, E. G. Buss, J. Wyban, and W. S. Hayward.** 1979. Ten genetic loci in the chicken that contain structural genes for endogenous avian leukosis viruses. Cold Spring Harbor Sym. Quant. Biol., vol. 44, in press.
10. **Bacon, L. D.** 1979. Origin of inbred and genetically defined strains of chickens, p. 607. *In* P. L. Altman and D. D. Katz (ed.), Inbred and genetically defined strains of laboratory animals. Federation of American Societies for Experimental Biology, Bethesda, Md.
11. **Baluda, M. A.** 1972. Widespread presence in chickens of DNA complementary to the RNA genome of avian leukosis virus. Proc. Natl. Acad. Sci. U.S.A. **69**:576-580.
12. **DiStefano, H. S., and R. M. Dougherty.** 1966. Mechanism of congenital transmission of avian leukosis virus. J. Natl. Cancer Inst. **37**:869-883.
13. **Duesberg, P., P. K. Vogt, K. Beeman, and M. Lai.** 1974. Avian RNA tumor viruses: mechanism of recombination and complexity of the genome. Cold Spring Harbor Symp. Quant. Biol. **39**:847-857.
14. **Ertl, H. H., L. E. Feinendegen, and H. J. Heiniger.** 1970. Iodine-125, a tracer in cell biology: physical properties and biological aspects. Phys. Med. Biol. **15**:447-456.
15. **Hayward, W. S., and H. Hanafusa.** 1975. Recombination between endogenous and exogenous RNA tumor virus genes as analyzed by nucleic acid hybridization. J. Virol. **15**:1367-1377.
16. **Hughes, S. H., F. Payvar, D. Spector, R. T. Schimke, H. L. Robinson, G. S. Payne, J. M. Bishop, and H. E. Varmus.** 1979. Heterogeneity of genetic loci in chickens: analysis of endogenous viral and nonviral genes by cleavage of DNA with restriction endonucleases. Cell **18**:347-359.
17. **Hughes, S., E. Stubblefield, F. Payvar, D. Engel, J. Dodgson, D. Spector, B. Cordell, R. Schimke, and H. E. Varmus.** 1979. Gene localization by chromosome fractionation: globin genes are on at least two chromosomes and three estrogen inducible genes are on three chromosomes. Proc. Natl. Acad. Sci. U.S.A. **76**:1348-1352.
18. **Karess, R. E., W. S. Hayward, and H. Hanafusa.** 1979. Cellular information in the genome of recovered avian sarcoma virus directs the synthesis of transforming protein. Proc. Natl. Acad. Sci. U.S.A. **76**:3154-3158.
19. **Ketner, G., and T. J. Kelly, Jr.** 1976. Integrated simian virus 40 sequences in transformed cell DNA. Analysis using restriction endonucleases. Proc. Natl. Acad. Sci. U.S.A. **73**:1102-1106.
20. **McClements, W., H. Hanafusa, S. Tilghman, and A. Skalka.** 1979. Structural studies on oncornavirus-related sequences in chicken genomic DNA. Two step analyses of *Eco*RI and *Bgl*I restriction digests and tentative mapping of a ubiquitous endogenous provirus. Proc. Natl. Acad. Sci. U.S.A. **76**:2165-2169.
21. **Neiman, P. E.** 1973. Measurement of endogenous leukosis virus nucleotide sequences in the DNA of normal avian embryos by RNA-DNA hybridization. Virology **53**:196-204.
22. **Robinson, H. L.** 1978. Inheritance and expression of chicken genes which are related to avian leukosis virus genes. Curr. Top. Microbiol. Immunol. **83**:1-37.
23. **Robinson, H. L., S. M. Astrin, and F. H. Salazar.** 1979. V-15_B, and allele of chickens for the production of a noninfectious avian leukosis virus. Virology **99**:10-20.
24. **Rosenthal, P., H. C. Robinson, W. S. Robinson, T. Hanafusa, and H. Hanafusa.** 1971. DNA in uninfected and virus-infected cells complementary to avian tumor virus RNA. Proc. Natl. Acad. U.S.A. **68**:2336-2340.
25. **Rubin, H.** 1957. Interactions between Newcastle disease virus (NDV), antibody and cell. Virology **4**:533-562.
26. **Skalka, A., P. DeBona, F. Hishinuma, and W. McClements.** 1979. Avian endogenous proviral DNA: analysis of integrated *ev* 1 and a related gs$^-$ chf$^-$ provirus purified by molecular cloning. Cold Spring Harbor Symp. Quant. Biol., vol. 44, in press.
27. **Southern, E.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503-518.
28. **Stehelin, D., H. E. Varmus, J. M. Bishop, and P. K. Vogt.** 1976. DNA related to the transforming gene(s) of avian sarcoma viruses is present in normal avian DNA. Nature (London) **260**:170-173.
29. **Tereba, A., M. M. C. Lai, and K. G. Murti.** 1979. Chromosome 1 contains the endogenous RAV-O retrovirus sequences in chicken cells. Proc. Natl. Acad. Sci. U.S.A. **76**:6486-6490.
30. **Tereba, A., L. Skoog, and P. K. Vogt.** 1975. RNA tumor virus specific sequences in nuclear DNA of several avian species. Virology **65**:524-534.
31. **Tooze, J. (ed.).** 1973. The molecular biology of RNA tumor viruses, p. 618-619. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
32. **Tsichlis, P. N., and J. M. Coffin.** 1980. Recombinants

between endogenous and exogenous avian tumor viruses: role of the C region and other portions of the genome in the control of replication and transformation. J. Virol. **33**:238–249.

33. **Varmus, H. E., J. M. Bishop, and P. K. Vogt.** 1973. Appearance of virus-specific DNA in mammalian cells following transformation by Rous sarcoma virus. J. Mol. Biol. **74**:613–626.
34. **Varmus, H. E., R. A. Weiss, R. R. Friis, W. A. Levinson, and J. M. Bishop.** 1972. Detection of avian tumor virus-specific nucleotide sequences in avian cell DNA. Proc. Natl. Acad. Sci. U.S.A. **69**:20–24.
35. **Vigne, R., M. L. Breitman, C. Moscovici, and P. K. Vogt.** 1979. Restitution of fibroblast-transforming ability in src deletion mutants of avian sarcoma virus during animal passage. Virology **93**:413–426.
36. **Wang, L. H., C. C. Halpern, M. Nadel, and H. Hanafusa.** 1978. Recombination between viral and cellular sequences generates transforming sarcoma virus. Proc. Natl. Acad. Sci. U.S.A. **75**:5812–5816.