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New Findings on the Congenital Transmission of Avian Leukosis Viruses

Abstract. *RAV-0, an endogenous avian leukosis virus, does not undergo congenital transmission in infected K28 chickens. In contrast, avian leukosis viruses of exogenous origin undergo highly efficient congenital transmission. The relative abilities of endogenous and exogenous viruses to undergo congenital transmission appear to be determined by the p27 capsid proteins of these viruses.*

Field strains of avian leukosis viruses (ALV's) have long been known to undergo efficient congenital transmission (*1*). These viruses replicate through the reproductive tract of the hen, with the highest numbers of budding virus occurring in the magnum- or albumen-secreting region of the oviduct (*2*). Developing embryos become infected and hatch into chicks whose immune systems recognize the virus as self (*3*). A persistent viremia is established with each generation of viremic hens spawning viremic progeny.

To study the biology of endogenous viruses, we have developed a randomly bred line of K28 chickens that is susceptible to endogenous virus infections (*4*). Endogenous viruses replicate well in K28 chickens, establishing levels of viremia comparable to those observed in K28 chickens infected with exogenous ALV's (*5*). As expected, K28 hens infected with exogenous ALV's shed high titers of virus into egg albumen. However, K28 hens infected with the endogenous virus RAV-0 did not shed virus into egg albumen (Table 1).

To define which region of the genome of endogenous viruses restricted the shedding of virus into eggs, we examined eight recombinants of endogenous and exogenous ALV's for their ability to undergo congenital transmission (Table 2). Four of these (RAV-60's) were generated during growth of exogenous viruses in cells that expressed replication-defective endogenous viruses (*ev* 3 or *ev* 9) (*6*). Two (NTRE-2 and NTRE-7) were generated during mixed infection of cells with an exogenous virus and an endogenous virus (*7*). The remaining recombinants (recALV's) were recovered from DNA constructions that recombined specific fragments of molecularly cloned proviral DNA's.

Tests for the congenital transmission of the recombinant viruses were performed on viremic K28 layers. Only layers with 10^5 or more infectious units of virus per milliliter of serum were examined for shedding of virus into eggs (*8*). The presence of virus in egg albumen was assayed by incubating albumen with antiserum to the group-specific antigens of ALV's and then testing for complement fixation (*9*). Most albumens containing virus scored positive at the highest dilution tested (1:16). Five of the eight recombinant viruses underwent efficient egg transmission while three did not (Table 2).

The recombinants were grouped as to whether or not they were shed into egg albumen (Table 3). The genomes of the recombinants were then scored for markers from their endogenous and exogenous parents. Most markers were oligonucleotides that were diagnostic for the endogenous or the exogenous parents of the recombinants (*6*, *7*). The host range of the recombinant was used as a marker for gp85 sequences, with N designating the subgroup E host range that is characteristic of endogenous ALV's (*10*). The electrophoretic mobility of the p27 protein was used as a marker for

Table 1. Congenital transmission of exogenous but not endogenous ALV's in K28 chickens. Albumens were collected from freshly layed eggs of K28 hens that had been intravenously inoculated at 1 day of age with approximately 1×10^6 infectious units of the indicated viruses. pRAV-1 is virus recovered from RAV-1 proviral DNA cloned into the Sac I site of pBR322. pRAV-0 is virus recovered from RAV-0 proviral DNA cloned into the Sal I site of pBR322. Sera were collected from laying hens, and virus was measured by assaying for the amount of particulate RNA-directed DNA polymerase. Under our assay conditions, 1 count/min is equivalent to approximately 100 infectious units of virus (*8*). ALV group-specific antigens were determined by assaying for complement fixation in reactions of egg albumen and antiserum to group-specific antigens (*9*). The group-specific antigens in egg albumen appeared to be in mature virus since they had the electrophoretic mobilities that are characteristic of the viral (p27, p19, p15, p12) rather than the precursor forms (Pr76, Pr66) of group-specific (gs) antigens (*14*, *15*). N.T., not tested.

Virus	Subgroup of virus	Number of hens	Viremia (infectious units per milliliter)	gs^+ albumens/albumens tested
		Exogenous		
RAV-1	A	2	N.T.	7/7
pRAV-1		1	2×10^7	4/4
tdPr-B	B	2	N.T.	4/4
		Endogenous		
RAV-0	E	27	N.T.	0/81
pRAV-0		5	1×10^6 to 5×10^6	0/15

p27, with X designating a p27 with the electrophoretic mobility characteristic of that of exogenous ALV's and N designating a p27 protein with an electrophoretic mobility characteristic of that of the p27° of endogenous ALV's (Fig. 1) (*11*).

All of the recombinants had the subgroup E host range of their endogenous parent and U3 sequences from their exogenous parent (Table 3). Thus, gp85 sequences that determine the host range and U3 sequences that contain the transcriptional control elements of ALV's do not determine whether these viruses undergo efficient congenital transmission. U5, p19, and gp37 sequences also do not appear to play a key role in congenital transmission, since recombinants that both did and did not undergo transmission were segregating for markers in these viral sequences. However, a direct correlation was found between the presence of the p27 marker of exogenous ALV's and the ability of a recombinant to be shed into egg albumen. The five recombinants with the exogenous p27 marker underwent efficient shedding into eggs, whereas the three recombinants with the endogenous p27° marker did not.

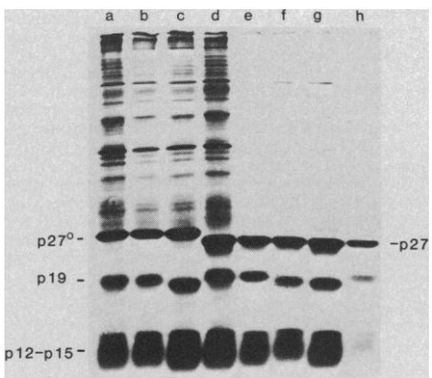

Fig. 1. Protein markers in the recombinant viruses. K28 cultures infected with RAV-0, the exogenous virus MCAV, and the indicated recombinant viruses were labeled with [^{35}S]methionine as described (*11*). Virus was recovered from the culture medium, dissociated, and fractionated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Isotopically labeled proteins were visualized by autoradiography. p27, p19, p12, and p15 are the major capsid proteins of ALV's. p 27° designates the characteristic size of the p27 of endogenous ALV's. p19 frequently exhibits altered sizes in recombinants of endogenous and exogenous ALV's (*11*). (Lane a) RAV-0, (lane b) NTRE-7, (lane c) NY202RAV-60, (lane d) NTRE-2, (lane e) NY201RAV-60, (lane f) NY203RAV-60, (lane g) NY204RAV-60, and (lane h) MCAV.

Table 2. Congenital transmission of recombinants of exogenous and endogenous ALV's. RecALVWF44 and 45 are viruses recovered from DNA constructs that substituted gp37-LTR sequences (Sal I–Sac I fragment) of pRAV-1 for the comparable fragment of pRAV-0 in pRAV-0. RecALVBr22 is virus recovered from a DNA construct of *gag-pol*-gp85 sequences (Sac I–Sal I fragment) of a lambda clone of NTRE-2 proviral DNA and gp37-LTR sequences (Sal I–Sac I fragment) of a lambda clone of NY203 proviral DNA. See text and legend to Table 1 for description of experiment and assays for viremia and group-specific (gs) antigens.

Parents of recombinant	Designation of recombinant	Number of hens	Viremia (infectious units per milliliter)	gs$^+$ albumens/albumens tested
RAV-1, *ev* 3	NY201RAV-60	5	1×10^6 to 8×10^6	20/20
RAV-1, *ev* 9	NY203RAV-60	1	1×10^7	4/4
RAV-2, *ev* 3	NY202RAV-60	3	4×10^5 to 1×10^6	0/14
RAV-2, *ev* 9	NY204RAV-60	1	1×10^7	4/4
tdPr-B, RAV-0	NTRE-2	3	8×10^5 to 6×10^6	12/12
	NTRE-7	5	2×10^5 to 8×10^6	0/15
pRAV-1, pRAV-0	recALVWF44, 45	3	4×10^5 to 2×10^6	0/11
λNY203, λNTRE-2	recALVBr22	4	5×10^5 to 2×10^6	10/10

Table 3. Genetic composition of recombinants. Markers for sequences in U5, p19, *pol*, gp37, and U3 were oligonucleotides that are characteristic of the endogenous or exogenous parent (*6*, *7*). The electrophoretic mobility of the p27 protein was used as a marker for p27 and p27° (see Fig. 1) and the subgroup of the recombinant as the marker for gp85. No markers were present for the p12 and p15 capsid proteins. N, marker from endogenous parent; X, marker from exogenous parent.

Recombinant virus	U5	p19	p27	*pol*	gp85	gp37	U3
			Transmitted				
NY201 RAU-60	X	X + N	X	X	N	X	X
NY203 RAU-60	X	X	X	X	N	X	X
NY204 RAU-60	X	N + X	X	N	N	X	X
NTRE-2	N	N	X	X	N	N + X	X
recALVBr22	N	N	X	X	N	X	X
			Nontransmitted				
NY202 RAU-60	X	N + X	N	X	N	X	X
NTRE-7	N	N	N	N	N	N	X
recALVWF44, 45	N	N	N	N	N	X	X

Since the complement fixation assay for group-specific antigens is a relatively insensitive assay for ALV's, the restriction on the congenital transmission of endogenous ALV's was examined by an assay that would detect a single infectious unit of virus. In this assay, fertile eggs were obtained from viremic layers infected with RAV-0 and NTRE-7. The former is our prototype endogenous virus and the latter is a recombinant that has the U3 sequences, and therefore high growth potential, of exogenous ALV's (*8*). Cells were cultured from embryos (10 days of gestation) and assayed at 1, 2, and 3 weeks of culture for the production of RAV-0 or NTRE-7 (*8*). No virus was produced by cultures obtained from 26 different embryos of six hens infected with RAV-0 and five infected with NTRE-7 (*8*). Since 3 weeks of culture is more than adequate for a single infectious unit of RAV-0 or NTRE-7 to grow to high titer, the embryos were virus-free (*8*). Thus, the restriction on the ability of RAV-0 and NTRE-7 to be passed by congenital infection in K28 chickens appears to be absolute.

The ability of ALV's to undergo congenital transmission in K28 does not correlate with the ability of these viruses to establish high titers of virus in serum (Tables 1 and 2). Thus, ALV's appear to have tissue-specific restrictions on their replication, with p27° viruses replicating well in tissues that shed virus into blood but poorly in reproductive tissue. The nature of the host factor that determines whether a p27° virus will or will not replicate in oviduct tissue is not known. Since the restriction appears to act on the major capsid protein of ALV's (Table 3), our hypothesis is that it prevents the opening of the virus capsid in infected cells.

Although there is no precedence for p27 affecting the replication of ALV's, there is precedence for the analogous protein of murine leukemia viruses (MuLV's) affecting virus growth. The p30 capsid proteins of endogenous MuLV's affect the efficiency with which these viruses replicate on inbred strains of mice (N-/B- tropism) (*12*). The N-/B-tropism of MuLV's is determined not only by the viral p30 protein but also by

host factors encoded by dominant alleles at the *Fv*-1 locus (*13*).

To our knowledge, these results are the first report of a viral capsid protein playing a critical role in the congenital transmission of a retrovirus. Whether capsid proteins affect the replication of other families of retroviruses in reproductive tissue is not known. However, since the ability to undergo efficient congenital transmission has survival value for exogenous but not endogenous viruses, the major capsid proteins for all exogenous and endogenous viruses may have undergone selection for their ability to ensure or restrict the replication of virus in reproductive tissue. If so, the capsid proteins of exogenous and endogenous viruses may provide genes that can be used to construct viruses that either will or will not undergo congenital transmission.

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Structure of 3′ Terminal Region of Type II Human T Lymphotropic Virus: Evidence for New Coding Region

Abstract. *The sequence of the 3′ terminus of the human T lymphotropic virus type II (HTLV-II) was determined and compared to the corresponding sequence of HTLV-I. The 1557-nucleotide-long sequence can be divided into a 5′ region that is not conserved between the two viruses, and a 3′, 1011-nucleotide-long region that is highly conserved and that corresponds precisely with a long open reading frame for both HTLV-I and -II. The proteins that could be encoded by these open reading frames have a molecular weight of about 38,000 and are closely related in primary amino acid sequence. The genomic structure in the 3′ region of HTLV was found to be similar to that of bovine leukemia virus.*

The human T lymphotropic viruses (HTLV) are a family of retroviruses that are associated with T-cell abnormalities (*1*). Isolates known as HTLV-I are associated with an aggressive form of adult T-cell leukemia or lymphoma (*1*). An infrequent isolate known as HTLV-II was first identified in a patient with a T-cell variant of hairy cell leukemia (*2*). Recently, some viruses collectively called HTLV-III were isolated from patients with the acquired immune deficiency syndrome (*3*).

The genomes of HTLV-I and -II differ from those of the nonacute retroviruses, which encode only the *gag*, *pol*, and *env* genes, in that they have an additional sequence that is approximately 1600 nucleotides long. This sequence is located between the 3′ end of the *env* gene and the 5′ end of the U3 region of the proviral long terminal repeat (LTR) (*4*).

Although this sequence occupies a position similar to the *src* gene in Rous sarcoma virus, it is not homologous to conserved mammalian genes and therefore differs from the oncogenes of transforming retroviruses (*4*). There is some evidence that this region contains a functional gene. Heteroduplex analysis of HTLV-I and -II reveals a conserved sequence about 1000 nucleotides long near the 3′ terminus of the genome (*5*). Spliced messenger RNA (mRNA) species that contain sequences that are unique to the 5′ end of the viral genome (U5 LTR sequences) and a portion of the 3′ sequence are observed in HTLV-infected cells and in some fresh tumor cells (*6*). Seiki *et al.* (*4*) note that several open reading frames occur within the 3′ sequence of HTLV-I.

To obtain a clearer understanding of the potential role of the 3′ region of HTLV, we determined the primary nucleotide sequence of the region located between the 3′ end of the *env* gene and the LTR of a cloned HTLV-II provirus, MO15A (*7*).

The nucleotide sequence of 1557 bases of the 3′ terminal region of HTLV-II is presented in Fig. 1. This sequence can be divided into two regions. One region, 546 nucleotides long, is located at the 5′ end of the sequence and has either no or very little similarity to the corresponding sequences in HTLV-I. For this reason we call this sequence the nonconserved region (NCR). A second region, 1011 nucleotides long, comprises the 3′ portion of this sequence. This sequence is very similar to that of HTLV-I and is identical at 765 of 1011 nucleotides (76 percent identity).

A new gene? The perimeters of the 1011 nucleotide sequence of the HTLV-II genome correspond precisely with a single long open reading frame capable of encoding a polypeptide 337 amino acids long. A corresponding sequence of HTLV-I also encompasses a single long open reading frame capable of encoding a polypeptide 357 amino acids long. We call the nucleotide sequence containing these long open reading frames the LOR region (nucleotides 566 to 1557 in HTLV-II) (Fig. 1).

The predicted amino acid sequences of both polypeptides are presented in Fig. 1. The potential proteins encoded by the LOR regions of HTLV-I and -II are of approximately the same length and are identical in 259 of 337 of the amino acids (77 percent identity). The degree of similarity of these two proteins is even more striking if conservative amino acid substitutions are considered (89 percent similar). The distribution of hydrophilic and hydrophobic regions of these proteins is remarkably similar (Fig. 2).

We also note the existence of a splice acceptor consensus sequence located at the 5′ end of the open reading frame (Fig. 1). Although no methionine codon occurs at the 5′ end of the open reading frames of HTLV-I and -II, a fusion protein synthesized from a spliced mRNA can be envisioned. Several other splice acceptor sequences occur within this reading frame from which smaller fusion proteins might also be made.

These observations suggest that the 3′ terminal region of HTLV contains a new gene that encodes a protein with a mo-