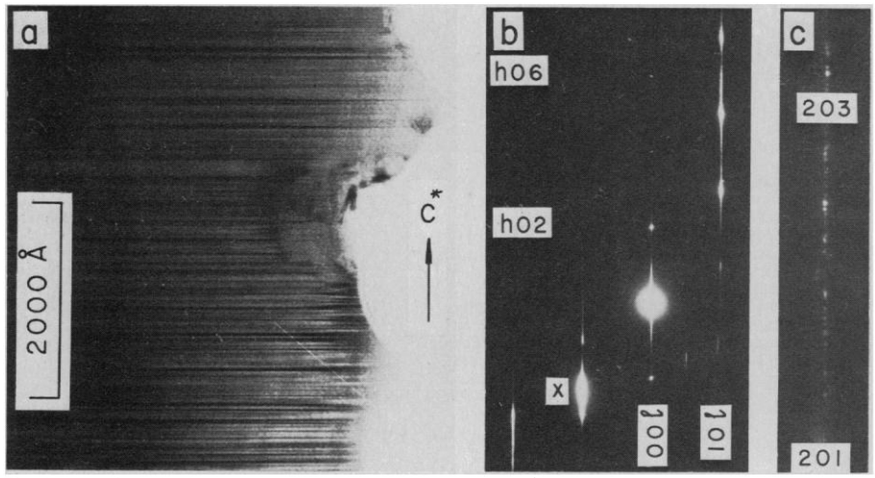

Fig. 2. (a) Bright-field micrograph of stacking faults in Pribram wurtzite. (b) Diffraction pattern of an area similar to that shown in (a); x is a diffuse maximum not related to a 2H reciprocal lattice point. (c) Enlargement of part of a 20ℓ row of another diffraction pattern showing diffraction maxima characteristic of a long-period polytype.

shown in Fig. 2b: such diffraction patterns are invariably eccentric since wurtzite does not cleave very readily on $\{110\}$. The reciprocal lattice rows with $h + k \neq 3n$ (10ℓ, 20ℓ, and so on) show continuous streaks, with diffuse maxima about 2H lattice points with $\ell = 2n + 1$, apparently consistent with the presence of random stacking faults. Other diffuse maxima on these rows (for example, x in Fig. 2b) are due to maximum diffraction contrast since they are displaced as the grains are tilted and correspond with bend contours in dark-field images. Bright-field images of grains parallel and subparallel to the a^*,c plane are crowded with diffraction fringes (Fig. 2a) which are normal to the diffraction streaks. One may image these fringes in dark field, using the diffraction streaks on reciprocal lattice rows with $h + k \neq 3n$ but not with reflections on other rows and they branch at thickness contours (*5*). Thus the fringes are clearly associated with stacking faults in the basal planes (*6*). The smallest fringe spacing observed is 20 Å, approximately equal to a 6H period, but the structural interpretation of this correlation may not be direct. The stacking fault density is particularly high, at least comparable to that of the so-called microtwins in inverted ZnS films (*7*), and its distribution is relatively even. The stacking faults are not terminated by partial dislocations, forming stacking faults in prism planes (*6*) or some other type of structural discontinuity, but appear to be continuous across the grains. Continuity is even maintained across embayments in crenulate grain boundaries, a clear indication that the stacking faults predate sample preparation.

On more detailed examination (Fig. 2c) the continuous diffraction streaks are sometimes seen to contain closely spaced diffraction maxima, the resolution of which varies markedly both between areas of a single diffraction pattern and between different diffraction patterns. Measurements on the diffraction pattern illustrated (Fig. 2c) indicate a periodicity relative to a 1H layer of 65, and measurements on five other patterns, all from different grains, give approximate periodicities of 100, 105, 130, 130, and 130, respectively. However, the accuracy of these latter data may be suspect because of poor contrast and limited continuity. It was clearly impossible to discriminate between hexagonal and rhombohedral patterns.

An obvious explanation for these diffraction maxima is that they represent ordered regions within a generally disordered 2H wurtzite matrix (*4*), each ordered region being a long-period wurtzite polytype directly analogous to the 594R polytype of SiC (*8*). The fact that the spacing and shape of the diffraction maxima do vary somewhat (Fig. 2c) may be attributed to one, or a combination of several, of the following factors: (i) long unit cells compared to the selected area aperture diameter and specimen thickness, (ii) incomplete long-range order, (iii) imperfect long-range order, and (iv) coherent scattering between the polytypic regions and the 2H wurtzite matrix. Theoretically, stacking faults are expected to be visible in both long-period polytypes and 2H wurtzite matrix, and these structurally distinct regions have not yet been resolved in bright- and dark-field electron optical modes. However, the apparently even and dense distribution of stacking faults in ordered regions coupled with the observation that the long-period diffraction maxima are superimposed on the 2H wurtzite pattern do suggest that structures of the Pribram polytypes are based on modulations of the 2H structural unit. These structures are quite unlike those of synthetic long-period polytypes, and this difference may be related to different thermal histories. The synthetic polytypes appear to develop during cooling after high-temperature crystal synthesis (*9*), whereas the Pribram wurtzite formed in a cavity in carbonate rock under geologically low-temperature conditions.

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1. The crystal structure of 2H wurtzite may be represented by the stacking sequence ABABABA . . . and that of sphalerite by ABCABCAB . . ., where A, B, and C each represent a single stacking unit; H indicates hexagonal symmetry and R (below) rhombohedral symmetry.
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Evolution of Type C Viral Genes: Origin of Feline Leukemia Virus

Abstract. *Reiterated gene sequences related to the RNA of feline leukemia virus (FeLV) are detected in all tissues of domestic cats and their close* Felis *relatives but not in more distantly related* Felis *species. Partially homologous viral gene sequences are found in rodent, and particularly rat, DNA. Together with the immunologic relationships observed between FeLV and endogenous rodent type C viruses, the results lead to the conclusion that FeLV-related genes were transmitted from a rodent to cat ancestor and have been perpetuated in the germ line of cats.*

Feline leukemia virus (FeLV) produces acute leukemia and lymphoma in domestic cats (*1*) and can be transmitted horizontally from animal to animal as an infectious disease (*2*). However, sequences partially related to the RNA genome of FeLV are

found in the cellular DNA of normal domestic cats (*3*), and are inherited as stable Mendelian units in various genetic crosses (*4*). Our studies show that FeLV-related gene sequences are found not only in the cellular DNA of specific pathogen-free domestic cats (*Felis catus*) but also in the DNA of three other closely related Felidae. More distant *Felis* species lack these genes, while the cellular DNA of rodents, and in particular that of rats, contains related virogene sequences. Thus, FeLV-related gene sequences have been genetically transmitted in four *Felis* species since their evolutionary divergence from a common ancestor. The absence of such sequences in other Felidae and the presence of related sequences in rodents suggests that FeLV-related genes were introduced into the *Felis* lineage after trans-species infection by a type C virus of rodent origin.

Table 1 shows that four cat species have gene sequences related to FeLV in their cellular DNA. These species include the domestic cat (*Felis catus*), the jungle cat (*Felis chaus*), the sand cat (*Felis margarita*), and the European wildcat (*Felis sylvestris*), all originating from the Mediterranean Basin. Other species of *Felis* from sub-Saharan Africa, Southeast Asia, and North or South America lack FeLV-related DNA sequences. Complete reassociation analyses indicate that the FeLV-related sequences are reiterated from seven to ten times per haploid genome (*4*), a gene frequency characteristic of endogenous type C virogenes of several other mammalian species (*5*). Similar data were obtained with the use of [^{3}H]DNA transcripts prepared from the RNA of several strains of FeLV (Gardner-Arnstein, Snyder-Theilen, and Rickard); these strains are closely related by nucleic acid hybridization (*6*).

The thermal stability of double-stranded DNA is an index of base-pair mismatching between the hybridized strands (*7*). The reductions in the thermal stabilities of hybrids formed between unique sequence, cellular DNA from the domestic cat and the DNA of 13 feline species are shown in Table 1. The cellular DNA's of all the Felidae are closely related (< 2.0°C thermal stability difference), consistent with their recent divergence from a common ancestor within the last 10 million years. On the basis of thermal stability data, those species that have FeLV-related gene sequences appear to be more closely related to one another than to those that lack the sequences. The absence of FeLV-related DNA sequences in most of the Felidae suggests, then, that these genes were acquired by cats subsequent to the initial Felidae divergence but prior to that of the four positive *Felis* species.

When cellular DNA's of other mammalian species were examined, sequences homologous to the FeLV [^{3}H]DNA probe were detected only in rodents (Table 1). The most significant degree of hybridization was seen with rat cellular DNA, while other rodents (mouse and hamster) contained more divergent sequences. No such

Table 1. Nucleic acid homology between nonrepetitive cellular DNA from the domestic cat, RD-114 and FeLV viral DNA, and the DNA from various species. Cellular DNA was extracted from various tissues of the species listed (*11*). [^{3}H]Thymidine-labeled nonrepetitive domestic cat cellular DNA was isolated and hybridized to the cellular DNA of the various species (*11*). The percent hybrid is the saturating normalized value obtained after digestion of the hybrids with S_1 nuclease. The actual final extent of domestic cat cell DNA hybridization to the cellular [^{3}H]DNA probe was 68 percent. The temperature at which 50 percent of the hybrids are dissociated (T_m) was 86°C for the homologous domestic cat • domestic cat hybrid; the ΔT_m is the difference in T_m between the other DNA • DNA hybrids and the T_m of the homologous hybrid. The ΔT_m values were derived from quadruplicate experiments in which hybrids were melted at 1°C intervals over a range of ± 5°C from the T_m. A [^{3}H]DNA transcript of the helper virus of the Gardner-Arnstein strain of feline sarcoma virus grown in dog thymus cells and of RD-114 virus grown in a bat lung cell line were hybridized to the DNA of the species listed. The genomes of viruses of the FeLV and RD-114 groups do not share any nucleic acid sequences in common (*3, 6*). The [^{3}H]DNA probes were synthesized from detergent-disrupted type C virus in the presence of actinomycin D (*15*) and contained 58 to 60 percent of their respective 70*S* viral RNA sequences at a [^{3}H]DNA : ^{32}P viral RNA molar ratio of 2.0 (*11*). The DNA • DNA hybridizations were performed at 65°C in the presence of 0.75*M* NaCl, and hybrids were detected with S_1 nuclease (*11*). The actual final extent of hybridization of the RD-114 and FeLV [^{3}H]DNA probes to domestic cat cellular DNA was 86 percent and 41 percent, respectively (*16*). The figures in italics indicate degrees of homology significantly above background level.

Species	Cellular [^{3}H]DNA		Percent hybrid		
			FeLV [^{3}H]DNA		RD-114 [^{3}H]DNA
	Percent hybrid	ΔT_m	S_1	HA*	S_1
	Carnivores, Felidae				
Felis					
Domestic cat (*F. catus*)†	100	0.0	*100*	100	*100*
European wildcat (*F. sylvestris*)	> 95	0.3	*98*		*95*
Sand cat (*F. margarita*)	> 95	0.2	*83*		*81*
Jungle cat (*F. chaus*)	> 95	0.5	*76*		*70*
Golden cat (*F. aurata*)	> 95	0.7	3		2
Serval (*F. serval*)	> 95	1.2	2		3
Leopard cat (*F. bengalensis*)	> 95	1.0	2		2
Fishing cat (*F. viverrina*)	> 95	1.4	3		4
Geoffroy's cat (*F. geoffroyi*)	> 95	1.2	2		2
Other cats					
Bobcat (*Lynx rufus*)	> 95	0.9	2		3
Lion (*Panthera leo*)	92	1.5	4		2
Jaguar (*Leo onca*)	90	2.0	2		2
Snow leopard (*Uncia uncia*)	91	1.9	2		3
	Other carnivores				
Mink (*Mustela vison*)	23	12.0	2		2
	Rodents				
Rat (*Rattus norvegicus*)	2		*12*	*41*	2
(*Rattus rattus*)	2		*11*	*37*	2
Mouse (*Mus musculus*)	3		*7*	*26*	2
(*Mus caroli*)	2		*6*	*24*	3
Hamster (*Cricetulus griseus*)	2		*5*	*20*	2
Guinea pig (*Cavia porcellus*)	2		1	4	2
Capybara (*Hydrochoerus* spp.)	1		2	4	2
	Primates				
Woolly monkey (*Lagothrix* spp.)	2		2	6	2
Baboon (*Papio hamadryas*)	2		2	4	*23*
Mangabey (*Cercocebus atys*)	3		2	4	*19*
Patas (*Erythrocebus patas*)	1		2	5	*18*
Chimpanzee (*Pan troglodytes*)	2		2	4	*10*
Human (*Homo sapiens*)	2		2	3	5
	Other mammals				
Pig (*Sus scrofa*)	2		2		2
Cow (*Bos taurus*)	2		2	4	2
Rabbit (*Oryctolagus cuniculus*)	2		3		3
Bat (*Tadarida brasiliensis*)	2		2	5	1

*DNA • DNA hybridizations were performed at 56°C in 0.50*M* NaCl and duplexes were detected on hydroxyapatite (*19*). †This cat was reared in a germfree environment at Merck Sharp & Dohme (West Point, Pa.). Cats from this colony have never been found to be positive for infectious feline leukemia virus.

Table 2. Antigenic cross-reactions between type C viral p30 proteins as measured by radioimmunoprecipitation. Purified p30 proteins (*20*) were labeled with ^{125}I to approximate specific activities of 5 $\mu c/\mu g$ and were titrated by a double antibody method (*21*) with antiserums to homologous and heterologous p30 proteins. Antibody titers are presented as the reciprocal of the serum dilution required for 20 percent binding of the labeled test antigens. The source of type C viruses was as described; RaLV and MuLV are endogenous rat and murine type C viruses, respectively (*9*). Numbers in italics represent antibody titers obtained in homologous precipitating systems.

^{125}I-labeled p30 protein	Antibody titer			
	Anti-FeLV	Anti-RaLV	Anti-MuLV	Anti-RD-114
FeLV (Rickard strain)	*400,000*	1,200	2,500	400
RaLV (CCL-38 virus)	40,000	*10,000*	5,000	500
MuLV (S2CL3 virus)	25,000	600	*60,000*	300
RD-114	1,200	< 100	300	*20,000*

sequences could be detected in rodents more distantly related to the rat (guinea pig and capybara). A comparable extent of homology is also observed between FeLV [^{3}H]DNA transcripts and the RNA's of various rat type C viruses. Table 1 also shows data obtained with the FeLV viral probe under less stringent hybridization conditions and with hydroxyapatite to detect duplexes. These techniques facilitate the detection of partial DNA hybrids that are more extensively mismatched (*8*). Although background values are somewhat higher, the partial homology between the FeLV viral probe and rodent, especially rat, DNA is clearly demonstrated.

Immunologic studies support the concept that cats were infected by viruses of rodent origin at a point in recent evolutionary history (*9*). Figure 1 shows that an antiserum to the reverse transcriptase of FeLV cross-reacts strongly with enzymes of endogenous rat type C viruses and less well with the polymerases of endogenous murine and hamster viruses. No significant inhibition was seen with the enzymes of endogenous porcine or baboon type C viruses. Like the reverse transcriptases, the p30 proteins of rodent, and particularly rat, type C viruses are immunologically related to each other and to FeLV (Table 2), indicating their derivation from a common ancestor. Other mammalian type C viruses, including the feline RD-114 group (*10*), are more distantly related by these criteria.

The homologies observed between feline leukemia viruses and the endogenous viruses of rodents are analogous to those seen with endogenous feline viruses of the RD-114 group and endogenous primate type C viruses (*11, 12*). Previous results have indicated that viruses of the RD-114 group were transmitted during the Pliocene to ancestors of the domestic cat from an ancestor of present-day Old World monkeys (*13*). The [^{3}H]DNA transcripts of RD-114 viral RNA hybridize to the cellular DNA of all Old World monkeys, while RD-114–related sequences are only found in the DNA of certain *Felis* species (Table 1). It is interesting that those cats which contain sequences related to RD-114 also contain FeLV-related genes, particularly since each group of viral genes has been derived from a distinctly different group of animals (primates and rodents).

The cellular control of FeLV and RD-114 virogenes appears to be quite different. Viruses of the RD-114 group are readily induced from "virus-negative" cat cell clones, but are generally restricted from replicating in cat cells (*14*). In contrast, viruses of the FeLV group have not been induced from cat cells in culture, but are able to replicate and readily cause disease in domestic cats. We propose that cat leukemia has resulted from the activation of genetically transmitted, rodent-derived virogenes that are rarely expressed in feral populations. In the domesticated cat, in urban areas and in multiple cat households where contact between animals is extensive and leukemia incidence is greatly elevated, the spread of those viruses no longer capable of being restricted by the cat is facilitated (*2, 22*).

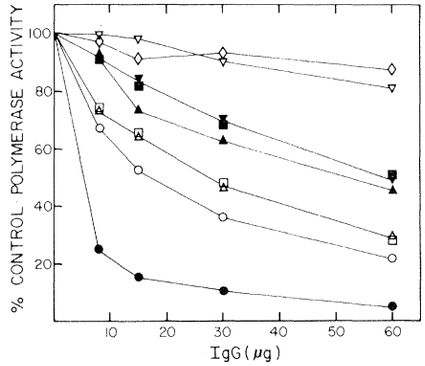

Fig. 1. Inhibition of type C viral reverse transcriptases using antiserum to the polymerase of FeLV. The conditions of the assay were as reported (*9*). Enzymes were obtained from: FeLV (●); the endogenous rat viruses RT21C (□), CCL-38 (○), and V-NRK (△); the endogenous hamster virus, CHO, released spontaneously by Chinese hamster cells (■); the endogenous murine viruses, S2CL3 (▼) and AT-124 (▲); the endogenous porcine virus, PK-15 (▽); and the endogenous baboon virus, M7 (◊).

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