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Transforming Genes of Retroviruses: Definition, Specificity, and Relation to Cellular DNA*

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A. Abstract

The oncogenic properties of sarcoma, acute leukemia, and lymphatic leukemia viruses are interpreted in terms of their genetic structures. Highly oncogenic sarcoma and acute leukemia viruses are shown to contain transforming *onc* genes which are different from the three virion genes (*gag, pol,* and *env*) essential for replication. Biochemical and genetic approaches to define *onc* genes are discussed. The hallmark of retroviral *onc* genes is shown to be a specific RNA sequence that is unrelated to essential virion genes. On this basis five different classes of *onc* genes can be distinguished in the avian tumor virus group alone: two of these, the *onc* genes of Rous sarcoma virus (RSV) and avian myeloblastosis virus (AMV), share one design. Their coding sequence is a specific RNA section which either replaces *env* [RSV(−), AMV] or maps adjacent to the 3′ end of *env* (RSV). Expression of this class of *onc* genes is mediated via subgenomic mRNAs containing sequences from the 5′ end of viral RNA spliced onto the *onc* gene coding sequences. The *onc* gene product of RSV has been identified as a 60,000-dalton phosphoprotein. Three other classes of *onc* genes, namely, those of the myelocytomatosis (MC29) subgroup of viruses, avian erythroblastosis virus (AEV), and Fujinami sarcoma virus (FSV), share another design. Their coding sequences are hybrids consisting of specific as well as of *gag* or *gag* and *pol* gene-related elements. The products of these *onc* genes, translated from full size genomic RNA, are hybrid proteins carrying *gag* or *gag* and *pol* determinants in addition to specific sequences. They are phosphorylated and range in size from 75,000 to 200,000 daltons. Since viruses with totally different *onc* genes can cause the same disease (namely, RSV, FSV, AEV, and MC29 cause sarcoma and AEV, AMV, or E26 and MC29 cause erythroblastosis), it is concluded that multiple mechanisms involving multiple cellular targets exist for sarcomagenic and leukemic transformation of the avian cell. Comparisons between viral *onc* genes of the RSV-design and in particular those of the hybrid design and *onc*-related chromosomal DNA sequences of the cell suggest qualitative differences. Hence viral *onc* genes are not simply transduced cellular genes, and cellular sequences related to viral *onc* genes appear not directly relevant to cancer. It follows that viral *onc* genes are unique and more than the sum of their parts related to cellular DNA and to replicative genes of retroviruses. We speculate that *onc* genes also may plan a role indirectly in cancers caused by lymphatic leukemia viruses, although these viruses are not known to contain such genes.

B. Introduction

Retroviruses cause sarcomas, carcinomas, acute and lymphatic leukemias, or no disease in animals (Gross 1970; Beard et al. 1973; Tooze 1973; Levy 1978; Jarrett 1978; Duesberg 1980; Essex 1980). Table 1 shows schematically the pathology of representative re-

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Table 1. Oncogenic properties of retroviruses

Viruses	Tumors in animals				Transformation in culture	
	Sarcoma	Carcinoma	Leukemia		Fibro-blast	Blood cell
			Acute	Lymphatic		
Sarcoma viruses						
RSV, RSV(−)						
FSV						
Mo-MuSV	+	−/+[a]	+[b]	−/+[c]	+	?
Ki-Ha-MuSV						
FeSV						
Acute leukemia class I						
Avian MC29 subgroup:						
MC29, MH2, CMII, OK10	+[d]	+[d]	+	−/+[c]	+	+
AEV						
Abelson MuLV						
Acute leukemia class II						
AMV, E26	−[e]	−[e]	+	+	−	+
Lymphatic leukemia						
Avian leukosis and						
Rous-associated						
viruses, tdRSV, RAV(O)	−[f]	−[f]	−	+/−	−	−
MuLV						
FeLV						

[a] Liver and kidney metastases have been reported for RSV (Gross 1970; Purchase and Burmester 1972). FeSV has been shown to cause melanocarcinomas (McCullough et al. 1972; Chen et al. 1980)

[b] Observed among other nontumorous diseases with Harvey, Kirsten, and Moloney MuSV (see text) (Scher et al. 1975; Ostertag et al. 1980) and rarely with RSV (Gross 1970; Purchase and Burmester 1972)

[c] Possibly due to helper virus (Gross 1970; Purchase and Burmester 1972; Graf and Beug 1978)

[d] Not observed with Abelson virus (Rosenberg and Baltimore 1980)

[e] Some (Beard 1973; Purchase and Burmester 1972), but not all (Moscovici 1975), stocks of AMV have caused carcinomas or sarcomas, perhaps due to other helper virus components

[f] Lymphatic leukemia viruses have been described to cause sarcomas and carcinomas at low frequency and after long latent periods (Gross 1970; Purchase and Burmester 1972). However, sarcoma- or carcinoma-causing variants have not been isolated

troviruses of the avian, murine, and feline tumor virus groups. The avian, murine, and feline sarcoma viruses predominantly cause sarcomas and transform fibroblasts in culture. The Harvey (Ha), Kirsten (Ki), and Moloney (Mo) murine sarcoma viruses (MuSV) and rarely avian Rous sarcoma virus (RSV) also cause erythroid leukemia (Gross 1970; Scher et al. 1975; Ostertag et al. 1980; Duesberg 1980). This has not been observed with avian Fujinami virus (FSV) (Lee et al. 1980). Feline sarcoma virus (FeSV) in addition to sarcomas also causes melanocarcinomas (McCullough et al. 1972; Chen et al. 1981). The avian acute leukemia viruses of the MC29 subgroup and erythroblastosis virus (AEV) that transform fibroblasts [therefore termed "class I" (Duesberg 1980)] and hematopoietic cells in culture have broad oncogenic spectra including sarcomas and carcinomas in addition to acute leukemias in the animal (Beard et al. 1973; Graf and Beug 1978). However, the fibroblast-transforming murine Abelson leukemia virus has not been reported to cause sarcomas and carcinomas (Rosenberg and Baltimore 1980). By contrast the avian acute leukemia viruses AMV and E26 that do not transform fibroblasts in culture [therefore termed "class II" (Duesberg 1980)] have rather specific oncogenic spectra in the animal, where they

cause myeloid and erythroid leukemias (Beard et al. 1973; Moscovici 1975; Graf and Beug 1978). The viruses listed thus far have in common that they transform quickly, within 1–2 weeks, and that transformation is an inevitable consequence of infection in susceptible animals. This implies that transforming *onc* genes are integral parts of the genomes of these viruses.

This appears not to be true for the majority of naturally occurring retroviruses, the lymphatic leukemia viruses. These are rather ubiquitous, nondefective viruses that often cause viremias but rarely and in particular not simultaneously cause leukemias (Gross 1970; Tooze 1973), as for example in chickens (Rubin et al. 1962; Weyl and Dougherty 1977), mice (Gardner et al. 1976; Levy 1978; Cloyd et al. 1980), or cats (Jarrett 1978; Essex 1980). The transformation-defective (td), *src*-deletion mutants of RSV have the same biologic (Biggs et al. 1972) and genetic properties (Wang et al. 1976) as the lymphatic leukemia viruses (Fig. 1). The RNA genome of these viruses contains a 3′terminal c-region and all three essential virion genes in the following 5′ to 3′ order: *gag* (for internal virion proteins or *g*roup-specific *a*nti*g*ens), *pol* (for RNA dependent DNA *pol*ymerase), and *env* (for *env*elope glycoprotein). The *c*-region has regulatory functions in the reverse transcription of viral RNA and in the transcription of proviral DNA (Fig. 1) (Wang et al. 1975; Wang 1978; Tsichlis and Coffin 1980). The endogenous, nondefective (containing all three virion genes) retroviruses of chicken, such as RAV(0) (Tooze 1973), and of mice, such as xenotropic viruses (Levy 1978; Cloyd et al. 1980), are inherited according to Mendelian genetics. These viruses probably never cause a disease directly and appear to differ from the more pathogenic lymphatic leukemia viruses in minor genetic elements, including the *c*-region that influences virus expression (Tsichlis and Coffin 1980; Lung et al. 1980; Cloyd et al. 1980). Since transformation is not or is only rarely a consequence of replication by any of these viruses and only occurs after considerable latent periods, these viruses may not contain authentic *onc* genes.

In the following we will describe the definition of *onc* genes of the rapidly transforming sarcoma and acute leukemia viruses. On this basis we will then ask whether the oncogenic specificity of some and the lack of specificity by other viruses is due to distinct *onc* genes or whether one *onc* gene can cause multiple forms of cancer. In addition, we review the question of the relationship between viral *onc* genes and cellular DNA. Finally, the question is addressed of how lymphatic leukemia viruses, which lack known *onc* genes, may cause cancer. The focus will be on avian tumor viruses, because their genetic structures are better defined than those of other viruses. This review extends two previous ones published recently (Duesberg, 1980; Bister and Duesberg 1980).

C. Definition of src, the onc Gene of RSV

The only *onc* gene of retroviruses for which nearly complete genetic and biochemical definitions are available is the *src* gene of RSV. In 1970 the *src* gene was formally distinguished from the three essential virion genes of nondefective RSV by the isolation of a mutant that was temperature-sensitive only in transformation but not in virus replication (Martin 1970) and by the isolation of nonconditional *src*-deletion mutants (Duesberg and Vogt 1970; Martin and Duesberg 1972). These transformation-defective *src* deletion mutants retain all virion genes and are physically and serologically like wild type RSV (Fig. 1) (Wang et al. 1975; Wang 1978). However the RNA of the wild type measures 10 kb (kilobases) whereas that of the tdRSV measures only 8.5 kb (Fig. 1) (Duesberg and Vogt 1970, 1973; Lai et al. 1973; Beemon et al. 1974; Wang 1978). On the basis of this difference *src* gene-specific RNA sequences were first defined by subtracting from the 10-kb RNA of RSV with the genetic structure 5′ *gag-pol-env-src-c* 3′ the 8.5-kb RNA of the isogenic *src* deletion mutant tdRSV with the genetic structure 5′ *gag-pol-env-c* 3′ (Fig. 1) (Lai et al. 1973). The 1.5 kb that set apart the wild type RSV from the *src* deletion mutant were shown to be a contiguous sequence that mapped near the 3′ end of viral RNA (Wang et al. 1975).

The *src* gene was independently defined by recombination analysis in which a *src*-deletion mutant with variant virion genes (5′ *gag″-pol″-env″-c* 3′) was allowed to recombine with a nondefective RSV. All sarcomagenic recombinants with variant virion genes had the genetic structure 5′ *gag″-pol″-env″-src-c* 3′) and hence had inherited the *src* gene (Beemon et al. 1974; Wang et al. 1976; Wang 1978) (see

also Fig. 1). It followed that the 1.5-kb sequence of RSV-specific RNA was necessary for transformation.

A major step towards proving that the 1.5-kb *src*-specific RNA sequence was also (almost) sufficient for transformation was ta-

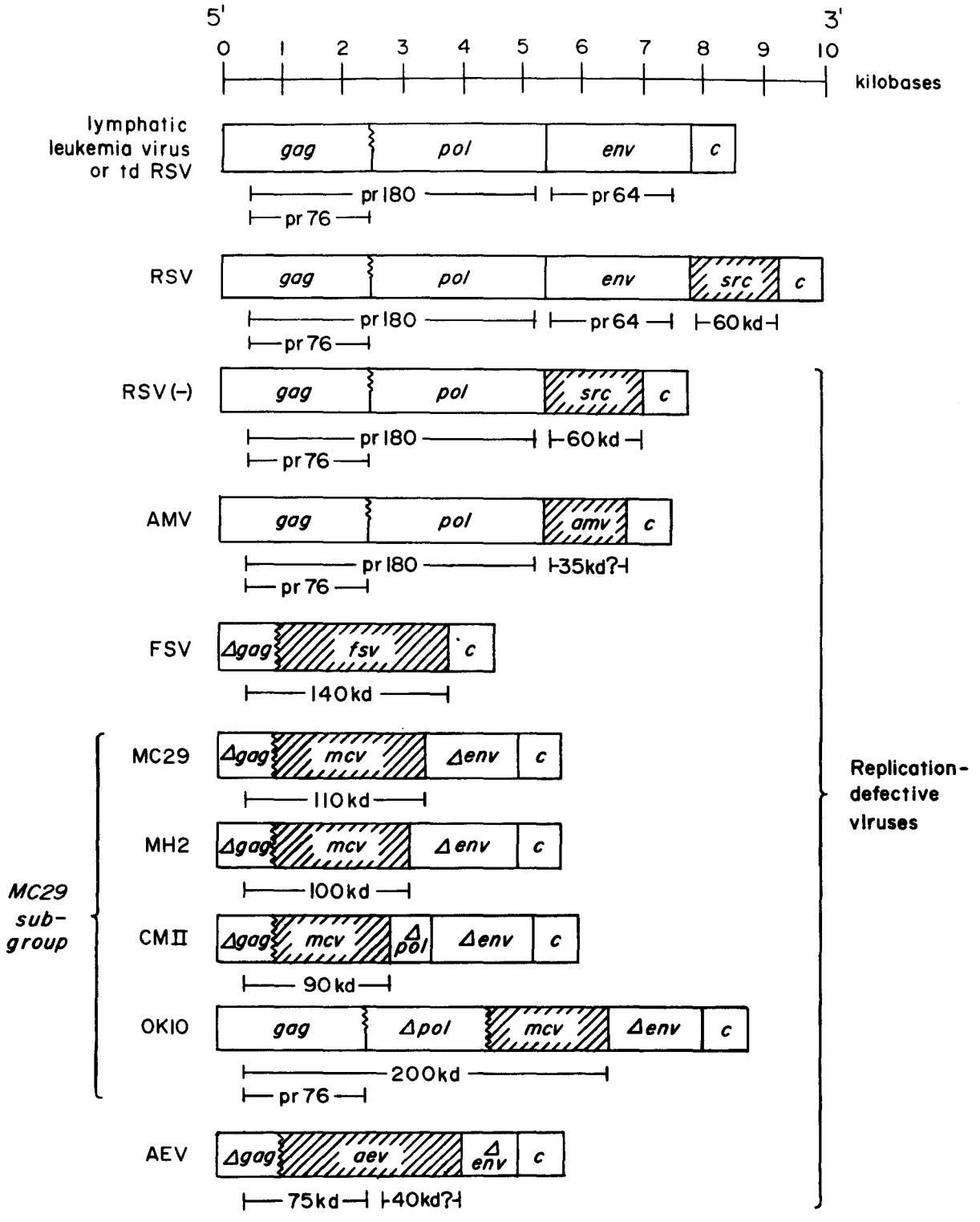

Fig. 1. Genetic structures of viruses of the avian tumor virus group. *White boxes* indicate map locations and complexities in kilobases of complete or partial (Δ) complements of the three essential virion genes *gag, pol* and *env* and of the noncoding *c* region at the 3' end of viral RNAs. *Hatched boxes* indicate location and complexities of specific sequence elements which are unrelated to essential virion genes and which define five distinct RNA subgroups within the avian tumor virus group. These specific sequences represent all *(src, amv)* or part *(fsv, mcv, aev)* of the coding sequences of the five different onc genes associated with avian tumor viruses. *Undulated lines* indicate that translation crosses and *half-undulated lines* that translation may cross borders between RNA sequence elements of different genetic origin. *Lines under the boxes* symbolize the complexities in kilodaltons of the protein products encoded by the respective RNA sequences

ken when a 60-kilodalton (kd) protein product was identified in RSV-transformed cells (Brugge and Erikson 1977) that was serologically unrelated to the *gag, pol,* and *env* virion proteins. Since the genetic complexity of the 1.5-kb RSV-specific RNA sequence and of the 60-kd *src* protein are about the same, the 1.5-kb RNA must encode most or all of the protein (Fig. 1). The same *src*-specific sequence has also been identified in a *env*-deletion mutant of RSV, termed RSV(−) (Table 1, Fig. 1) (Wang et al. 1976), and recently also in a gag^-, pol^-, and *env*-defective sarcomagenic deletion mutant of RSV (Martin et al. 1980). Transformation by the gag^-, pol^-, and *env*-defective RSV is definitive proof that the *src* gene (but not the *src*-specific RNA sequence by itself) is sufficient for transformation. Expression of the *src* gene of RSV involves a mRNA which also includes sequences derived from the 5′ and 3′ ends of virion RNA that are shared with *src*-deletion mutants of RSV (Mellon and Duesberg 1977). This implies that the *src*-specific sequence of RSV defined by deletion and recombination analysis does not act independently and may by itself not be sufficient for transformation.

Since the definition of the *src* gene of RSV most other acutely transforming retroviruses have been shown to contain specific sequences unrelated to essential virion genes. Such sequences appear to be the hallmark of highly oncogenic viruses, and they represent most or at least part of their *onc* genes (see below). To date the oncogenic retroviruses are the only class of viruses which have *onc* genes that are nonessential for virus replication. The only known function of these genes is their oncogenicity.

D. Identification of the Genome and Definition of the onc Genes of Replication-Defective Oncogenic Viruses

The definition of the *onc* genes of highly oncogenic viruses other than RSV is less advanced than that of *src*. This is because all highly oncogenic retroviruses, with the exception of RSV, lack essential virion genes and consequently are replication defective. The genetic phenotype of most defective sarcoma and acute leukemia viruses is: gag^-, pol^-, env^-, onc^+ (Fig. 1) (Tooze 1973; Bister and Vogt 1978; Graf and Beug 1978). Due to this phenotype classical deletion and recombination analysis cannot be used to define *onc* genes as was the case with *src*. An *onc* deletion of such a virus (i.e., gag^-, pol^-, env^-, onc^-) would obviously be undetectable by classical techniques measuring viral gene expression. Likewise the lack of secondary markers would complicate or prevent recombination analysis of *onc* genes of defective viruses.

I. Genome Identification

Defectiveness also complicates identification of the viral RNA genomes. The genome of a nondefective retrovirus is essentially identified by extracting the RNA from purified virus. Since the defective virus only replicates if complemented by a nondefective helper virus, usually a lymphatic leukemia virus, it is obtained as a complex containing defective as well as helper viral RNAs. Moreover, the two RNAs are replicated at unpredictable ratios, usually favoring the RNA of nondefective helper virus. The RNA of defective and helper virus was first separated by electrophoretic analysis of viral RNAs, which typically yields one larger RNA species measuring 8 to 9 kb and a smaller one measuring 4 to 7 kb (Maisel et al. 1973; Duesberg et al. 1977). By its absence from nondefective helper viruses, the small RNA species was shown to be necessary for transformation (Maisel et al. 1973; Duesberg et al. 1977).

Subsequent biochemical analyses directly identified the smaller RNA species, as the genome of the defective virus, which is consistent with its low complexity (Duesberg 1980; Bister and Duesberg 1980). The larger RNA species was shown to be the RNA of nondefective helper virus, whose size is invariably 8 to 9 kb as dictated by the requirement for complete *gag, pol,* and *env* genes in nondefective helper viruses (see Fig. 1 for examples).

II. Defining Transformation-Specific Nucleotide Sequences by Subtraction from the RNA of Defective Transforming Virus Sequences Shared with Nondefective Helper Viruses

Given the RNA of defective transforming virus, it can be asked whether a sequence unrelated to essential virion genes is present. Moreover in the avian system it can be asked

whether this sequence is related to the genetically defined *src*. Experimentally this is accomplished by comparing the RNAs (or proviral DNAs) of defective and helper virus by various nucleic acid scopes, including hybridization of defective viral RNA with cDNA of helper viruses, comparative fingerprinting of RNase T_1-oligonucleotides, or comparison of proviral DNA fragments generated by a given restriction enzyme. By these methods two classes of sequences are distinguished in the RNAs of defective transforming viruses: helper virus related and specific sequences (Duesberg 1980). In all cases examined the specific sequences form contiguous internal map segments which are flanked by helper virus related terminal map segments (Fig. 1) (Mellon et al. 1978; Bister and Duesberg 1980; Duesberg 1980).

Although biochemical subtraction of helper virus related sequences from the RNA of a defective, transforming virus is formally analogous to deletion analysis, the subtracted (shared) RNA is not the genome of a viable deletion mutant (cf. Fig. 1). Therefore, it can only be inferred but cannot be deduced from this type of analysis that the resulting specific sequence of the defective oncogenic virus (hatched in Fig. 1) is necessary for transformation.

III. Genetic Evidence That Specific and gag-Related RNA Sequences of Avian Class I Acute Leukemia and Fujinami Sarcoma Viruses Are Necessary for Oncogenicity

Genetic evidence was used to determine whether the specific sequences of defective transforming viruses are necessary for transformation. Since due to the absence of selective markers other than *onc*, suitable recombinants have not as yet been prepared in the laboratory, different isolates of closely and distantly related avian acute leukemia viruses were used as substitutes of recombinants. The RNAs of these viral isolates were compared to each other by electrophoretic size analysis, by hybridization with cDNAs of various avian tumor viruses, and by mapping RNase T_1-resistant oligonucleotides (Duesberg et al. 1977; Bister et al. 1979; Roussel et al. 1979; Duesberg et al. 1979; Bister et al. 1980a; Bister and Duesberg 1979, 1980).

Such comparisons show that four different isolates of avian acute leukemia viruses, whose oncogenic spectra are closely related (Table 1) (Beard et al. 1973; Graf and Beug 1978), namely, MC29, MH2, CMII, and OK10, also have closely related genetic structures (Fig. 1). The hallmark of each viral RNA is an internal, helper virus unrelated sequence of about 1.5 kb (Duesberg et al. 1977, 1979; Bister et al. 1979; Bister and Duesberg 1980; Bister et al. 1980a; Roussel et al. 1979). Because the specific sequences of the four viruses are closely related and because the sequence was first identified in MC29 virus, it has been termed *mcv*. The *mcv* sequence is the structural basis for the classification of the four viruses into the MC29 subgroup of avian RNA tumor viruses (Bister et al. 1979; Bister and Duesberg 1980; Bister et al. 1980a; Duesberg 1980).

The size and the oligonucleotide composition of the *mcv* sequences appears to vary between approximately 1.5 and 2 kb in different viral strains (Fig. 1) (Duesberg et al. 1979; Bister et al. 1979; Bister et al., 1980 a). Based on oligonucleotide complexity the largest *mcv* sequence appears to be that of MC29 virus and the smallest one either that of CMII or of MH2 (Bister et al. 1980a). In MC29, CMII and MH2 the *mcv* sequence ist flanked at the 5′ end by a partial (Δ) *gag* gene, termed Δ*gag*, and in OK10 by a complete *gag* followed by a Δ*pol* (Fig. 1). It is not clear as yet whether the Δ*gag* sequences of MC29, MH2, and CMII have the same complexities.

At the 3′ end the *mcv* sequence of MC29 and OK10 is flanked by a Δ*env* gene, and that of CMII, by Δ*pol* (Fig. 1) [*env* sequences are present in MH2 RNA, but the RNA has not been analyzed sufficiently to determine whether its *mcv* sequence borders at Δ*env* (Fig. 1) (Duesberg and Vogt 1979)].

These comparisons of the four MC29-subgroup viral RNAs show that: (1) The 5′ parts of their Δ*gag* or *gag* sequences and most, but not all, of their *mcv* sequences are highly conserved; (2) their *env*-related sequences are related by hybridization but variable if compared at the level of shared and specific T_1-oligonucleotides (Bister et al. 1979; Duesberg et al. 1979; Bister et al., 1980a), and (3) they may have optional sequences such as the 3′ half of *gag* and the Δ*pol* at the 5′ end of *mcv* in OK10 or the Δ*pol* at the 3′ end of *mcv* in CMII (Fig. 1). There are probably optional parts of the

mcv sequence itself, because its size appears to vary in different viral strains. It would follow that most of *mcv* is an essential specific correlate and *Δgag* an essential, nonspecific (because it is shared with helper virus and other defective viruses; Fig. 1) correlate of viral oncogenicity.

These genetic analyses are confirmed and extended if one includes AEV (Bister and Duesberg 1979) and FSV (Lee et al. 1980). Each of these viruses has a genetic structure similar to the viruses of the MC29 subgroup, with a *Δgag* sequence at the 5′ end and internal specific sequences, termed *aev* and *fsv* (Bister et al. 1980a), which are unrelated to essential virion genes, to *src*, to *mcv*, and to each other (Fig. 1). Thus, these viruses form an analogous series of defective transforming viruses. It follows that the internal specific sequence of each of these viruses is necessary but probably not sufficient for transformation, since oncogenicity of each of these viruses also correlates with a highly conserved *Δgag*. Hence the *onc* genes of these viruses appear to be genetic units consisting of *gag*-related and specific RNA sequences.

IV. Nonstructural, gag-Related Proteins Define Genetic Units of Helper Virus Related and Specific RNA Sequences, in Class I Avian Acute Leukemia and Fujinami Sarcoma Virus

Each of the class I avian acute leukemia viruses as well as FSV code for gag-related nonstructural phosphoproteins ranging in size from 75 to 200 kd (Fig. 1) (Bister et al. 1977; Bister et al. 1979; Hayman et al. 1979a,b; Bister and Duesberg 1980; Lee et al. 1980; Ramsay and Hayman 1980; Bister et al. 1980a). That these proteins are coded for by *gag*-related as well as specific sequences of viral RNA was deduced from in vitro translation of viral RNAs of known genetic structure (Mellon et al. 1978; Lee et al. 1980) and from peptide analyses of these proteins (Hayman et al. 1979a,b; Kitchener and Hayman 1980). This directly supports the view that the specific sequences of these viruses are not independent genetic units (Fig. 1). They function together with at least the 5′ part of *gag* (or all of *gag* and part of *pol* in OK10) as one genetic unit (Fig. 1) (Mellon et al. 1978; Bister and Duesberg 1980; Lee et al. 1980; Bister et al. 1980a). To indicate that translation crosses the border between *gag*-related or between *gag-Δpol*-related and specific sequence elements, these borders were drawn as undulated lines in Fig. 1, also indicating that their exact location is uncertain. Given the very similar oncogenic spectra of these viruses and assuming transforming function of these proteins, we deduce that the size differences among the 90-, 100-, 110-, and 200-kd *gag*-related proteins of CMII, MH2, MC29, and OK10 directly confirm the point made above on the basis of RNA analysis, i.e., that the *Δgag/gag-Δpol-mcv* units include optional elements (see Fig. 1).

Moreover the structure of the genetic unit coding for the 200-kd protein of OK10 which contains a complete *gag* and a *mcv* sequence which replaces only a part of *pol* (Fig. 1) is of particular interest regarding the role of *gag*-related sequences in these proteins. Based on analogy with *pol* gene expression by nondefective viruses which proceeds via a *gag-pol* precursor protein (Fig. 1), the OK10 protein could also be processed into a product that contains only *Δpol* and *mcv*. The fact that such a protein is not found in infected cells (Ramsay and Hayman 1980; our unpublished observations) suggest again that the *gag*-related portion is essential for the function of this protein. The optional nature of the *pol* sequence in OK10, already evident from its lack in other MC29 subgroup proteins, is underscored by the fact that it includes the *pol* sequences that map at the 3′ end of *mcv* in CMII which are not part of the CMII protein (Fig. 1) (Bister et al. 1979, 1980a). Because the genetic units of the MC29 subgroup viruses that read *Δgag-mcv* or *gag-Δpol-mcv* share conserved 5′ *gag* elements and most of *mcv* but differ in optional, internal sequences, it has been proposed that these genes and their protein products have two essential domains, one consisting of the conserved *gag*-related, the other of the conserved *mcv*-related sequences (Bister et al. 1980a).

Since the known proteins coded for by the class I acute leukemia viruses do not account for genetic information of the 3′ half of the viral RNAs, it cannot be excluded that 3′ terminal sequences are also necessary for transformation. Nevertheless the variability of the 3′ terminal sequences, both in terms of oligonucleotide composition and in relationship to *env* and *pol* genes (Fig. 1), as well as the lack of evidence for protein products synthesi-

zed in infected cells argue that these sequences may not be translated. It was hypothesized, therefore, that these sequences may not play a direct role in transformation (Duesberg et al. 1979; Bister et al.1980a; Bister and Duesberg 1980).

In contrast, all genetic information of FSV, which has a genetic structure that is similar to that of class I acute leukemia viruses (Fig. 1), can be accounted for in terms of one known viral protein. Moreover, if one assumes that transformation requires a viral protein and that the viral RNA is translated in only one reading frame, one may argue that in the case of FSV the *Δgag-fsv* sequence is not only necessary but also sufficient for transformation, since the genetic complexities of the 4.5-kb FSV RNA and of the 140-kd protein encoded by *Δgag-fsv* are about the same (Fig. 1).

V. The onc Gene of Avian Myeloblastosis (AMV) and E26 Virus, Two Avian Acute Leukemia Viruses of Class II

The AMV and E26 are acute leukemia viruses which fail to transform fibroblasts and cause no sarcomas and possibly no carcinomas, signaling a unique class of *onc* genes (Beard et al. 1973; Moscovici 1975; Graf and Beug 1978). Until recently the analysis of AMV and related viruses has been slow, because infectious virus typically contains a large excess of nondefective helper virus. This has been changed by the discovery of defective AMV particles which are released by AMV-transformed nonproducer myeloblasts. Such particles contain a 7.5-kb viral RNA and are infectious if fused into susceptible cells together with helper virus (Duesberg et al. 1980). Consistent with the ability of AMV to produce defective virus particles, the RNA was found to contain a complete *gag* and *pol* gene, and nonproducer cells contain 76-kd *gag* and 180-kd *gag-pol* precursor proteins (Fig. 1). However there is no evidence for *gag* or *gag* and *pol* related nonstructural proteins in AMV-transformed cells (Duesberg et al. 1980). Between *pol* and a unique 3′ terminal *c*-region AMV contains a specific *amv* sequence of about 1.5 kb that is unrelated to those of any other acutely transforming avian tumor virus except E26 (Fig. 1) (Duesberg et al. 1980). It appears that the genetic structure of AMV resembles closely that of RSV(−) (Fig. 1). From this genetic structure it may be expected that the *amv* sequence codes possibly for a specific protein unrelated to *gag* and *pol* genes by a mRNA similar to that coding for the *src* protein of RSV (Mellon and Duesberg 1977). It would be expected that this protein has a transforming function. Preliminary evidence indicates that a 35-kd protein is translated in vitro from AMV RNA (Fig. 1) (Lee and Duesberg, unpublished work).

E. Two Distinct onc Gene-Designs

The *onc* genes described here have – as far as defined – two different designs: those with a coding sequence that is specific and unrelated to essential virion genes, for example, the *src* gene of RSV and possibly the *onc* gene of AMV, and those with a coding sequence that is a hybrid of genetic elements derived from essential virion genes and specific sequences, for example, the *onc* genes of MC29, AEV, and FSV. The specific sequences of the hybrid *onc* genes are all inserted at their 5′ ends adjacent to partially deleted *gag* or *pol* genes (Fig. 1). By contrast the specific sequences of the *onc* genes, whose coding sequences lack genetic elements of essential virion genes, either replace *env* genes [RSV(−), AMV] or are inserted between *env* and the *c*-region (RSV) (Fig. 1). For convenient reference one design is referred to as "RSV design" and the other as "MC29 design" of *onc* genes according to the originally identified prototypes.

The two *onc* gene designs also differ in their mechanism of gene expression: the hybrid *onc* genes, whose specific sequences replace *gag* or *pol* genetic elements, are probably translated from genomic viral RNA into *gag* or *gag* and *pol* related proteins like the pr76 *gag* or pr180 *gag-pol* proteins of nontransforming viruses that they replace (Fig. 1). However, there is no evidence that the hybrid gene products are subsequently processed. By contrast the *src* gene of RSV and probably the *amv* sequence of AMV, which replace *env* or are inserted downstream of *env*, are translated from subgenomic mRNAs like the *env* genes of nontransforming viruses (Fig. 1) (Mellon and Duesberg 1977; Hayward 1977; Duesberg et al. 1980; Lee and Duesberg, unpublished work; Gonda and Bishop, personal communication). Hence the mechanism of gene expression of the two different *onc* designs closely follows that of the

5′ most virion gene that they partially or completely replace (Fig. 1).

To determine whether the *gag*-related elements of the hybrid *onc* genes are indeed essential for the probable transforming function of their proteins as our analyses suggest, it would be necessary to find transforming viruses in which the specific sequences of hybrid *onc* genes are not linked to *gag* or *pol* sequences. Conversely it would be interesting to know whether *src* or the *amv* sequence would have a transforming function if inserted into *gag* or *pol* genes.

It is thought that the 60-kd *src* gene product functions catalytically, probably as a phosphokinase (Erikson et al. 1980; Bishop et al. 1980), although there is evidence that kinase activity may not be the only function of the *src* gene product (Rübsamen et al. 1980; Bishop et al. 1980).

By contrast the function of the *gag*-related proteins of avian acute leukemia and Fujinami sarcoma viruses, of the Abelson MuLV, and of the feline sarcoma viruses may not be solely catalytic. Although a kinase activity again appears as a candidate for a catalytic function of these proteins, this has not been demonstrated in each of these proteins. It appears associated with the *gag*-related proteins of some strains of Abelson virus (Witte et al. 1980) and, with some uncertainty, also with the proteins of the Gardner and Snyder-Theilen strains of feline sarcoma virus (Reynolds et al. 1980) but has not been found in some avian viruses with *onc* genes of MC29 design (Bister et al. 1980b) and in the McDonough strain of feline sarcoma virus (Van de Ven et al. 1980). It is possible that these *onc* gene products have in addition to a possible catalytic function a structural function involving their *gag*-related elements. Analogous to the function of virion *gag* proteins, the *gag* portions of the nonstructural proteins of these viruses may function by binding to specific cellular and also to intracellular viral nucleic acid sequences. This specific binding may represent a regulatory function of a cellular catalytic activity or perhaps of a yet to be discovered catalytic activity of the *gag*-related proteins. This function would then correspond to one of the two domains diagnosed in the proteins of the MC29 subgroup viruses described above.

The consistent difficulties in isolating temperature-sensitive *onc* mutants of these viruses that respond fast to temperature shifts (unpublished experiments and personal communications) support the view that *onc* genes of the MC29 design may have a structural function.

F. Multiple onc Genes: Multiple Mechanisms and Multiple Targets of Transformation

Despite some insufficiencies in the definition of *onc* genes of defective viruses, it is clear that multiple, at least five different classes of specific sequences *(src, mcv, aev, fsv,* and *amv)* and hence probably five different *onc* genes exist in the avian tumor virus group alone. The number will increase if other viruses are analyzed and if viruses of other taxonomic groups are included. Some of these *onc* genes cause specific cancers in the animal: for example, RSV and FSV, which cause predominantly sarcomas, and AMV and E26, which cause specifically leukemias affecting myeloid or erythroid precursor cells or more primitive stem cells depending on the host [chicken or quail (Moscovici and Löliger, personal communication)]. Other *onc* genes like those of MC29 and AEV may in addition to acute leukemias cause sarcomas and carcinomas (Table 1).

The fact that different *onc* genes vary in specificity yet may cause the same cancer argues against a unique mechanism to transform a given class of differentiated cells. For example RSV, FSV, MC29, AEV, and even Kirsten MuSV (Galehouse and Duesberg 1976) all may cause sarcomas in birds and can also transform mammalian fibroblasts (not tested for FSV) (Quade 1979), although they contain totally different *onc* genes. Likewise, AEV, MC29, and E26 and AMV may cause erythroblastosis (Table 1) (Beard et al. 1973; Graf and Beug 1978), although their *onc* genes, except for those of AMV and E26 (Duesberg et al. 1980), are different. It is concluded that multiple mechanisms, involving multiple *onc* genes and *onc* gene products and presumably multiple cellular targets, exist for sarcomagenic and leukemogenic transformation. The fact that different *onc* genes cause the same cancers or that one *onc* gene may cause multiple cancers argues against the hypothesis that the transforming proteins of these viruses closely resemble specific cellular differentiation proteins and that transformation is a consequence of a competition between

a specific viral transforming protein and a specific related cellular counterpart (Graf et al. 1980).

The overlap among the oncogenic spectra of different *onc* genes suggests that different *onc* genes either interact with different specific cellular targets or that *onc* genes interact with nonspecific targets. A unique target for a given form of cancer would fail to explain why different *onc* genes may cause the same disease and why in some cases one *onc* gene may cause different cancers. The nature of cellular targets for viral transformation remains to be elucidated; it is believed to include factors determining susceptibility to virus infection and replication as well as intracellular substances that interact directly with viral transforming proteins.

G. On the Relationship Between Viral onc Genes and Cellular Chromosomal Sequences

The helper virus unrelated specific sequences of acutely transforming avian, murine, and feline viruses have been shown to have closely related cellular counterparts (Scolnick et al. 1973, 1975; Tsuchida et al. 1974; Frankel and Fischinger 1976; Stehelin et al. 1976b; Spector 1978a; Frankel et al. 1979; Sheiness and Bishop 1979; Hughes et al. 1979a,b; Souza et al. 1980; Oskarsson et al. 1980). This has lent support to the hypothesis that normal cells contain viral *onc* genes and that viral *onc* genes are transduced cellular genes (Huebner and Todaro 1969; Stephenson et al. 1979; Bishop et al. 1980). If correct, this hypothesis would predict paradoxically, that normal cells contain a number of viral or cellular *onc* genes that apparently are not subject to negative selection. (At present this number is around a dozen and is going up as more *onc* genes are defined.) Thus normaly would be an admirable effort of cellular suppression of endogenous *onc* genes. Although their cellular relatives are even less well defined than most viral *onc* genes themselves, enough is known about them to deduce that viral *onc* genes are not in the cell but that some or most of their coding sequences have related counterparts in cellular DNA.

1. One example is *src* of RSV: The only form in which the specific sequence of RSV has ever been shown to have transforming function is if it is part of the viral *src* gene. As such, this sequence is expressed via a mRNA that shares 5′ leader and 3′ terminal *c*-region sequences with other virion genes (Mellon and Duesberg 1977). These virion sequences are not found in all veterbrates said to contain *src*-related sequences (Spector et al. 1978a) except in some strains of chicken. Moreover in chicken *src*-related and endogenous virion gene-related sequences are not located on the same restriction fragments of cellular DNA (Hughes 1979b) nor on the same chromosomes (Hughes 1979a). In addition the cellular *src*-related mRNA and DNA sequences appear not to be colinear with those of RSVs (Wang et al. 1977; Spector et al. 1978b; Hughes et al. 1979b). Hence concrete qualitative differences set apart the *src* of RSV and its relatives in normal vertebrate cells.

2. Another example of a close relationship between a viral *onc* gene and cellular alleles is the case of MuSV: Most of the helper virus unrelated 1.5-kb sequence of MuSV has a closely related, perhaps identical counterpart in the cell (Oskarsson et al. 1980; Blair et al. 1980). However, molecularly cloned "MuSV-specific" DNA from the cell or from MuSV can only transform cultured mouse fibroblasts if it is first linked with (presumably noncoding) terminal sequences from MuSV or helper MuLV. Again concrete qualitative differences exist between the *onc* gene of MuSV and related DNA sequences of the cell. Moreover transformation by these modified MuSV-related sequences from the cell is abortive, and no infectious virus is recovered. Thus transformation by this kind of DNA and by infectious virus may prove not to be the same, although they appear indistinguishable based on the fibroblast assay.

3. The transforming genes of viruses which appear to be hybrids of structural and nonstructural viral genetic elements provide even more convincing evidence that viral *onc* genes and their cellular relatives are not the same thing: Although it has been shown that most but not all vertebrate cells contain sequences related to the helper virus unrelated part of MC29 and AEV, the helper virus-related elements of these viruses, in particular *gag*-related elements, do not have the same distribution and are not found in the same cells (Sheiness and Bishop 1979; Roussell et al. 1979). Hence *gag*-related sequences, thought to be an essential element of the *onc* genes of MC29 and AEV, are not part of the cellular

sequences related to those genes. Moreover, the cellular sequences related to the 3-kb AEV-specific RNA sequence have recently been shown to be distributed over a 15-kb DNA segment that includes AEV-related and AEV-unrelated sequences (J. M. Bishop, personal communication). Likewise, the cellular DNA sequence, related to the Abelson murine leukemia virus-specific RNA sequence of 3 kb (Shields et al. 1979), has been shown to be distributed over a 12-kb DNA segment that must include Abelson virus unrelated sequences (Goff et al. 1980). Hence in these cases proviral DNA and related cellular DNA sequences are not colinear. It follows that the genetic units of class I acute leukemia viruses that consist of *gag*-related and specific RNA sequences (Fig. 1) have no known counterparts in normal cells.

It is concluded that viral *onc* genes of the RSV design and in particular those of the MC29 design are different from related sequences present in the cell. The *onc* genes of the RSV design, like *src* and possibly *amv* (Fig. 1), may share most but possibly not all of their coding sequences with cellular homologs but differ from cellular relatives in essential regulatory elements. The *onc* genes of the MC29 design differ from cellular counterparts in coding (*gag* and *pol*-related sequences) as well as regulatory elements. Consequently, the cellular relatives of most viral *onc* genes are probably not present in the cell as functional *onc* genes as has been postulated (Huebner and Todaro 1969; Bishop et al. 1980) and hence are probably not directly relevant to transformation.

Instead these cellular sequences may be relevant to the archaeology of viral *onc* genes. Viral *onc* genes probably have been generated by rare transductions of cellular sequences by nondefective viruses. To generate *onc* genes of the RSV design transduction must have involved illegitimate recombination with nondefective virus. In the case of Moloney MuSV specific deletions of the parental nondefective virus also had to occur (Dina et al. 1976; Donoghue et al. 1979; Chien et al. 1979; Blair et al. 1980). Until the cellular *src*-related sequence is characterized directly, it remains unclear whether in the case of RSV the coding sequence of *src* was transduced unchanged or after alteration when RSV was generated.

Both transduction, involving again illegitimate recombination, as well as specific deletions of virion genes (see Fig. 1) must have been necessary to generate the *onc* genes of the MC29 design from cellular and viral genetic elements. Such events are much less likely to occur than, for example, the transduction by phage lambda of a functional galactosidase gene. It is noted that experimental evidence compatible with the transduction of cellular sequences has led to the hypothesis that viral transformation is the product of enhancing the dosage of endogenous cellular *onc* genes by homologous equivalents from exogenous viruses (Bishop et al. 1980). We submit that sequence transduction is not synonymous with the transduction of unaltered gene function which would be a necessary corollary of the gene dosage hypothesis. It would appear that viral *onc* genes are unique and more than the sum of their parts related to cellular DNA and replicative genes of retroviruses.

H. The Role of onc Genes in Carcinogenesis

Highly oncogenic viruses with *onc* genes such as those described here have only been isolated in relatively few cases from animal tumors (reviewed in Gross 1970; Tooze 1973; Duesberg 1980). By contrast leukosis and lymphatic leukemia viruses have been isolated from many viral cancers, in particular from leukemias (see above) (Gross 1970; Tooze 1973). Thus paradoxically the lymphatic leukemia viruses which lack known *onc* genes appear to be more relevant to viral carcinogenesis than retroviruses with known *onc* genes.

However, it has been argued that *onc* genes also play a role in carcinogenesis caused by lymphatic leukemia viruses (Duesberg 1980). These *onc* genes may derive from endogenous, defective retrovirus-like RNAs known to exist in some normal cells (Duesberg and Scolnick 1977; Scolnick et al. 1979) or from cellular genes acquired by processes involving illegitimate recombination and specific deletions (compare genetic structures shown in Fig. 1). The necessity for such a secondary event to occur would explain the poor correlation between the distribution of lymphatic leukemia viruses and cancers in animals (see above). The failure to find *onc* genes in most retroviral cancers may then reflect technical difficulties. These include the lack of suitable probes to

detect viral RNA as the only characteristic viral structural component or nonstructural proteins as the only characteristic products of defective transforming viruses. Moreover, detection of a putative defective-transforming virus will be complicated by the fact that the ratio of defective-transforming to nondefective helper virus is low in typical stocks of defective helper virus complexes (Duesberg et al. 1977; Bister and Duesberg 1979; Duesberg et al. 1979; Lee et al. 1980). Thus, the analysis of viral *onc* genes may prove to be less academic than it appears at present – it may provide the tools and concepts necessary to understand all retroviral cancers.

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