Anatomy of the RNA and Gene Products of MC29 and MH2, two Defective Avian Tumor Viruses Causing Acute Leukemia and Carcinoma: Evidence for a New Class of Transforming Genes

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Abstract

The RNA species of the defective avian acute leukemia virus MC29 and of the defective avian carcinoma virus MH2 and of their helper viruses were analyzed using gel electrophoresis, fingerprinting of RNase T_1-resistant oligonucleotides, RNA-cDNA hybridization and in vitro translation. A 28S RNA species, of 5700 nucleotides, was identified as MC29- or MH2-specific. MC29 RNA shared 4 out of about 17 and MH2 RNA at least 1 out of 16 T_1-oligonucleotides with several other avain tumor virus RNAs. In addition MC29 and MH2 RNAs shared 2 oligonucleotides which were not found in any other viral RNA tested. 60% of each 28S RNA could be hybridized by DNA complementary to other avian tumor virus RNAs (group-specific) but 40% could only be hybridized by homologous cDNA (specific). *Src* gene-related sequences of Rous sarcoma virus were not found in MC29 or MH2 RNA. The specific and group-specific sequences of MC29, defined in terms of their T_1-oligonucleotides, were located on a map of all T_1-oligonucleotides of viral RNA. Specific sequences mapped between 0,4 and 0,7 map units from the 3' poly(A) end and group-specific sequences mapped between 0 and 0,4 and 0,7 and 1 map units. The MC29-specific RNA segment was represented by 6 oligonucleotides, two of which were those shared only by MC29 and MH2 RNAs. In vitro translation of MC29 RNA generated a major 120000 dalton protein and minor 56000 and 37000 dalton proteins. The 120000 dalton protein shared sequences with the proteins of the avian tumor viral *gag* gene, which maps at the 5′ end of independently replicating viruses. Since a *gag* gene-related oligonucleotide was also found near the 5′ end of MC29 RNA, we propose that the 120000 MC29 protein was translated from the 5′ 60% of MC29 RNA. It would then include sequences of the defective *gag* gene as well as MC29-specific sequences.

Since both MC29 and MH2 lack the *src* (sarcoma) gene of Rous sarcoma virus, it is concluded that they contain a distinct class of transforming (*onc*) genes. We propose that the specific sequences of MC29 and MH2 represent

all, or part of, their *onc* genes because the *onc* genes of MC29 and MH2 are specific and represent the only known genetic function of these viruses. If this proposal is correct, the *onc* genes of MC29 and MH2 would be related, because the specific RNA sequence of MC29 shares 2 of 6 oligonucleotides with MH2. It would also follow that the 120000 dalton MC29 protein is a probable *onc* gene product, because it is translated from MC29-specific (and group-specific) sequences and because both MC29- and MH2-transformed cells contain specific 120000 and 100000 dalton proteins, respectively.

Introduction

MC29 and MH2 are avian RNA tumor viruses that cause acute leukemia, carcinoma and also transform fibroblasts in culture [1,2,3,4,5,6]. This oncogenic cell transformation is due to a transforming gene, termed *onc* [7], which has not been defined genetically or biochemically. Both viruses require a helper virus for replication because they are defective in all three replicative genes of the avian tumor virus group *gag* (for internal group-specific antigen), *pol* (for DNA polymerase) and *env* (envelope glycoprotein) [7]. The viral genome was identified as a 28S RNA species of 5700 nucleotides because it is absent from pure helper virus and because the sequence of 28S RNA remains conserved when propagated with different helper viruses [8.9.10.11]. Hybridization with DNAs complementary to avian tumor virus RNAs (cDNAs) have distinguished two sets of sequences in each RNA. One set comprises 60% of the RNA which is related to other, independently replicating members of the avian tumor virus group including nondefective Rous sarcoma virus (ndRSV) [8–11]. This set is termed group-specific and probably represents defective replicative genes as well as conserved regulatory and structural elements [8,9,10]. The second set comprises the specific sequences of MC29 and MH2 RNA, which represent 40% of each RNA and is a likely candidate for the *onc* gene of these viruses. Since neither MC29 nor MH2 RNA contains sequences related to the *onc* gene of ndRSV, which is termed *src*, it has been suggested that these viruses contain distinct *onc* genes [8–11]. These and similar studies on avian [9–15] and murine [16–18,23] viruses have suggested that within a given RNA tumor virus family, transforming genes may differ whereas replicative genes are relatively conserved.

It is the purpose of this study to biochemically define the *onc* genes of MC29 and MH2 in order to structurally and functionally compare them to each other and to the *src* gene of RSV. The *src* gene of ndRSV has been unambiguously defined by analyses of *src*-deletion mutants and *src*-recombinants as a sequence of about 1500 nucleotides that segregates with sarcomagenicity [13,15,19]. Moreover the *src* gene was mapped near the 3′ end of viral RNA [14,15,19] and appears to be translated into a protein of 60000 daltons [20,21]. Due to their defectiveness in all three replicative genes [4,5,6] *onc*-deletion mutants and recombinants of MC29 and MH2 would lack biologically detectable genetic markers in replicative genes. Further, the specific sequences of MC29 are not expected to recombine readily with other

avian tumor viruses lacking them, because analysis of tumor virus recombination has demonstrated that efficient recombination only occurs between closely related, allelic sequences [22]. Therefore, the approach that was used to define *src* of ndRSV cannot yet be used to define the *onc* genes of MC29 and MH2 or to define the *onc* genes of other defective transforming viruses.

In this paper we describe an alternate, more biochemical, approach to the definition of the *onc* genes of MC29 and MH2. We identify (for MC29 and MH2) and locate (only for MC29) on the viral 28S RNAs strain-specific and group-specific sequences, we then investigate, in the case of MC29, the proteins encoded by these sequences with an interest in identifying a protein involved in cell transformation. Preliminary work has been described [9–11] and recently a more complete comparison of MH2 and MC29 has been published [40].

Results

MC29 and MH2 Contain Specific 28S RNA Species which Share Specific Oligonucleotides

A 28S RNA species that is physically and chemically distinct from the 34S RNA of the helper virus has been found in several defective virus-helper virus complexes of MC29 and of MH2 [8–11]. To demonstrate that the 28S RNA species in each virus complex was specific to the defective transforming MC29 or MH2 virus, different MC29 and MH2 pseudotypes have been investigated for the presence of 28S RNAs. It can be seen in Fig. 1 that three different MC29 pseudotypes, MC29 (ring-neck pheasant virus [RPV]), MC29 (MC-associated virus of subgroup A [MCAV-A]) and MC29 (MCAV-B) each contained a 28S RNA species in addition to the 34S RNA of the respective helper virus. The same was true for the MH2 pseudotypes, MH2 (MH2AV of subgroups A and C [A+C]) and MH2 (RAV-7) (Fig. 1). By contrast, the RNA of helper viruses RPV [8], MCAV-A, MH2AV-A+C and RAV-7 contained only a 34S RNA species (not shown). It was concluded that different pseudotypes of MC29 as well as of MH2 each share physically indistinguishable 28S RNA species.

We have investigated the large RNase T_1-resistant oligonucleotides of the 28S RNAs of MC29 and MH2 as specific diagnostic markers of each viral RNA. This is experimentally complicated because owing to their defectiveness these viruses only replicate in the presence of a helper virus. Hence viral RNA is a mixture of defective and helper virus RNAs, the ratio of which may vary with a given infected culture [8]. If 28S RNA is present at equal or higher concentration than 34S RNA in this mixture, it can be detected and isolated by preparative electrophoresis as is shown in Fig. 1. However, 28S RNA prepared in this fashion is still contaminated by degraded 34S RNA, the degree of contamination depending on the relative amount of 34S RNA present in the mixture and on the integrity of each RNA species. Degraded

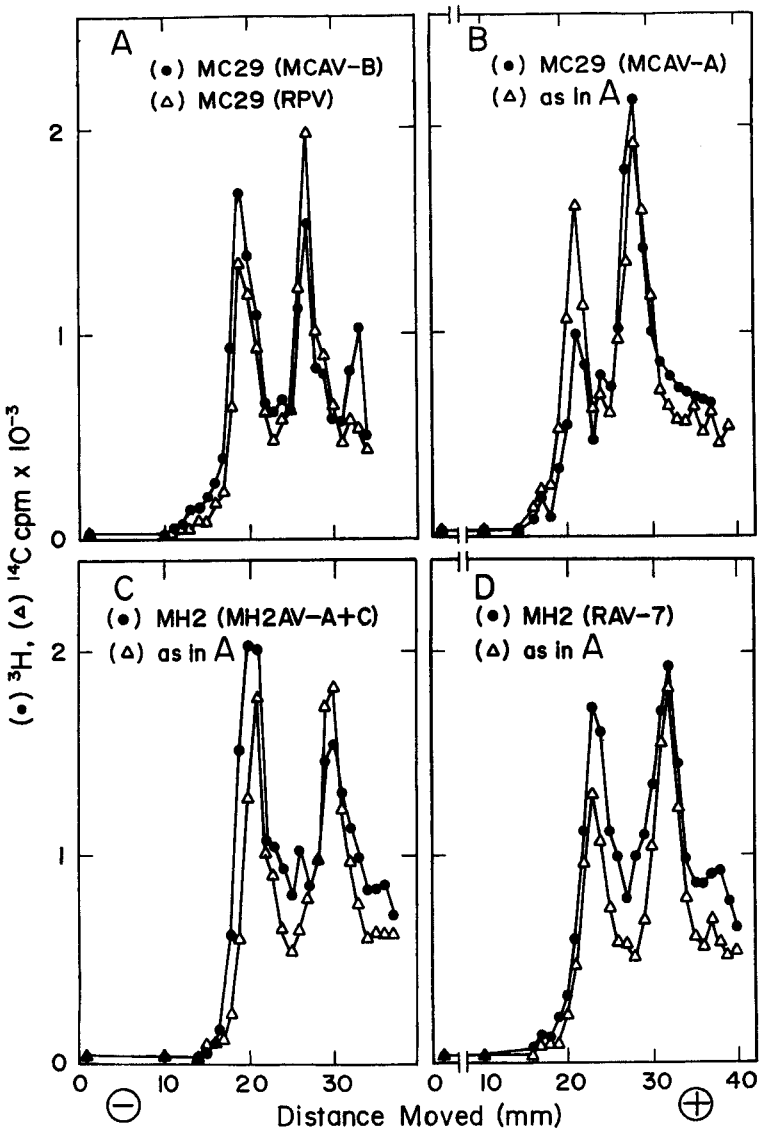

Fig. 1. The RNA monomers of different avian acute leukemia virus MC29-pseudotypes and of different avian carcinoma virus MH2-pseudotypes after electrophoresis in 2% polyacrylamide gels. Preparation of viral RNA and conditions for electrophoresis have been described [8]. Each RNA was electrophoresed with MC29 (ring-neck pheasant virus of subgroup F [RPV]) RNA standards containing a 28S MC29 and a 34S RPV RNA species [8]. (A) RNA of MC29 (MCAV-B). This pseudotype of MC29 was propagated on chick embryo fibroblasts and was obtained from C. Moscovici, who had received it via R. Smith from J. Beard. (B) RNA of MC29 (MCAV-A). This pseudotype of MC29 was also propagated on chicken fibroblasts and was obtained from C. Moscovici, who had received it from K. Bister and P. K. Vogt. (C) RNAs of MH2 (MH2AV-A and C) and (D) of MH2 (RAV-7). These pseudotypes of MH2 were prepared by one of us (P. K. Vogt) and had been propagated on chicken macrophage cultures

34S RNA that has the size of 28S RNA would coelectrophorese with intact 28S RNA of MC29 or MH2. Thus in order to distinguish 28S RNA-specific oligonucleotides from oligonucleotides derived from fragmented 34S RNA present in a given pool of 28S RNA, we have fingerprinted both RNA species. By subtracting from the 28S RNA oligonucleotides that the 34S and 28S RNAs have in common, we arrived at a minimal estimate of 28S RNA-specific oligonucleotides. A complete catalogue of 28S RNA-specific oligonucleotides must also identify those oligonucleotides that the 28S RNA

of a defective virus may have in common with the 34S helper virus RNA. Determination of such oligonucleotides was approached by analyses of 28S RNA pools that are little contaminated by 34S RNA (as was the case for RNA from MC29 (RPV) propagated by the quail Q8-cell line [8]) or by identifying specific oligonucleotides from different 28S RNA pools of a given defective virus that was propagated with different helper viruses.

Fingerprint analysis of 28S MC29 RNA prepared from MC29 (RPV) is shown in Fig. 2A and that of 34S RPV RNA has been published previously [8]. A comparison indicates that of all the T_1-oligonucleotides numbered in Fig. 2A, 28S MC29 RNA shares three, i.e., nos. 4, 9 and C with RPV RNA (8, 9, Table 1). Since the relative concentration of no. 4 was lower than that of all others in Fig. 2A and varied with different preparations of 28S MC29 RNA, we conclude that it was derived from contaminating RPV RNA (therefore it is in parentheses in Fig. 2A). Henceforth, the oligonucleotides of 28S RNA pools that are thought to derive from contaminating helper virus RNA are parenthesized in the Figures and Tables. Oligonucleotides shared with helper viruses that are present at equimolar ratios are believed to be integral parts of the 28S MC29 RNA shown in Fig. 2A. This was directly proven in the case of oligonucleotide no. 9. This oligonucleotide is a conserved element of the *gag* gene of other avian tumor viruses, which maps near the 5′ end of viral RNAs [27, 28]. If this oligonucleotide were present in a 28S RNA pool of MC29 (RPV) RNA as part of a RPV fragment, this fragment would have to include the 5′end of the 34S RNA but would lack the 3′ poly(A) end. Therefore only the poly(A)-tagged species of a 28S RNA pool of MC29 (RPV) RNA had been fingerprinted in Fig. 2A. The result that oligonucleotide no. 9 was present indicates that it was an integral part of 28S MC29 RNA. Its presence at a slightly lower than equimolar concentration in poly(A)-selected 28S RNA (Fig. 2A) was expected, because the 5′ end of the RNA would be preferentially lost in a poly(A) selection (compare Figs. 2A and 3A below). Analyses of the RNase A-resistant fragments of the large T_1-oligonucleotides of the 28S and the 34S RNA species of MC29 (MCAV-B) shown in Figs. 2B and C have not yet been completed. Preliminary results indicate that the 28S RNA species share several, large oligonucleotides with 28S MC29 RNA isolated from MC29 (RPV). These homologous oligonucleotides were given the same numbers in Fig. 2A and B. In addition, the 28S RNA species of MC29 (MCAV-B) contained oligonucleotides which appeared to have chromatographic counterparts in the 34S helper virus RNA of MC29 (MCAV-B) shown in Fig. 2C. Some of these probably derived from contaminating 34S helper RNA. In addition 28S MC29 RNA may also have acquired some helper viral oligonucleotides by recombination.

To identify MH2 RNA-specific oligonucleotides, 28S RNA pools from MH2 (MH2AV-A and C) and from MH2 (RAV-7) were compared to each other and to those of their 34S helper virus RNAs (Fig. 2E-H) as described above for MC29 RNA. MH2-specific oligonucleotides are numbered without parentheses in Fig. 2E, F and Table 2. Further work is necessary to determine whether some of the oligonucleotides shared by 28S MH2 and 34S MH2AV-

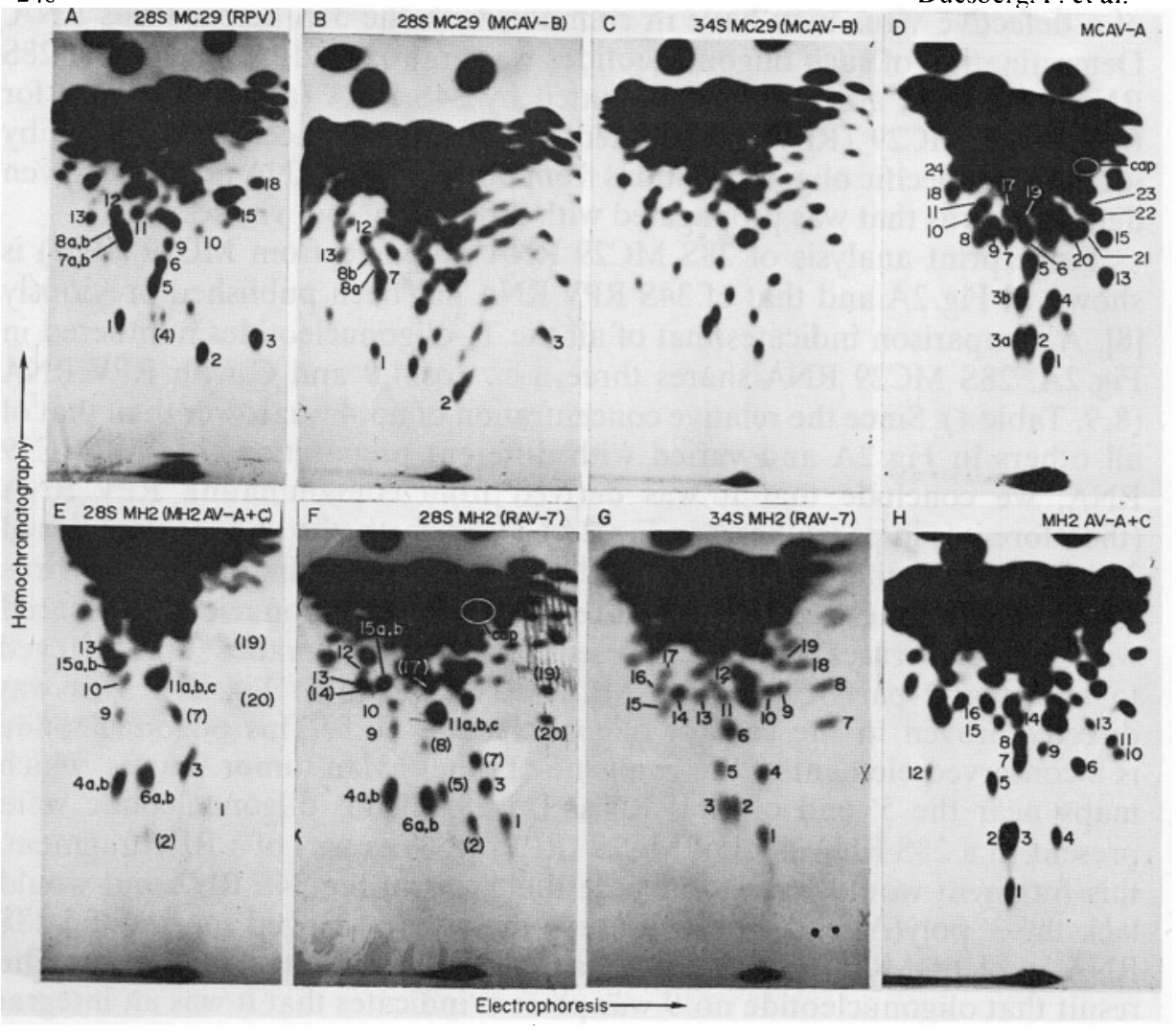

Fig. 2. Autoradiographs of RNase T_1-digested viral [^{32}P] RNA components after 2-dimensional electrophoresis-homo-chromatography (fingerprinting). Preparation of viral RNA components and conditions of fingerprinting have been described [8, 14]. Numbers identify large RNase T_1-resistant oligonucleotides, or spots consisting of more than one oligonucleotide. Numbers of oligonucleotides of 28S RNA pools which are probably derived from degraded 34S helper virus RNA are parenthesized. Cap designates the 5′ terminal capped oligonucleotide. The following RNAs were fingerprinted: (A) The poly(A)-containing 28S MC29 RNA prepared electrophoretically from MC29 (RPV) RNA as in Fig. 1 and chromatographed on oligo(dT)-cellulose [14]. (B) the 28S RNA of MC29 (MCAV-B), electrophoretically prepared from MC29 (MCAV-B) RNA as for Fig. 1. (C) the 34S MCAV-B RNA electrophoretically prepared from MC29 (MCAV-B) as in (B). (D) the 60–70S RNA of MCAV-A prepared from virus propagated in chicken fibroblasts. (E) the 28S RNA of MH2 (MH2AV-A and C), prepared as for (A, B). (F) the 28S RNA of MH2 (RAV-7) also prepared as for (A, B). (G) the 34S RAV-7 RNA prepared from MH2 (RAV-7) RNA as described for (C) and (H) the 50–70S RNA of MH2AV-A and C. A mixture of these two helper viruses was propagated in chicken fibroblasts

A and C or 34S RAV-7 RNAs are integral parts of 28S MH2 RNA or are derived from degraded helper virus RNA. Analysis of 28S RNA was hampered by difficulties with propagating sufficient radioactive virus in chicken macrophages [5] for biochemical analyses of RNA and because virus from transformed chicken or quail fibroblast cultures contained, in over thirty cases tested, > 3 times more 34S than 28S RNA, and was thus unsuitable for studying 28S RNA.

Table 1. Composition of T_1-oligonucleotides of 28S MC29 RNA[a]

28S MC29	RNase A digestion products	28S MH2[b]	MH2AV-A&C[b]	MCAV-A[b]	PR-B[b]	RPV[b]
Spot No.[c]				Spot No.		
1	2U, 3C, G, 2 (AC), (AU), 2 (AAC)					
2	7U, 7C, G, (AC), 3 (AU), (AAC), (AAU)					
3	8U, 2C, G, (AAC), <1 (AAAN)					
(4)	5U, 8C, 3 (AC), (AU), (AAC), (AAG)					1
5	3U, 5C, 2 (AC), (AU), (AAU), (AAAC), (A_4G)					
6	2U, 8C, 3 (AU), (AG), (AAAU)					
7a	2U, 6C, G, 2 (AC), (AU), (AAC)					
7b	3U, 8C, (AC), (AAG)	9				
8a	2U, 2C, G, 2 (AC), (AAAC)					
8b	2U, 4C, (AC), (AG), (AAU), (AAAC)	10				
9	5U, 6C, (AAG), (A_4C)	11a,b,c?		5a,b	8	5
10	3U, 2C, 2 (AU), (AG), (AAU)					
11	2U, 4C, G, 2 (AC), (AU), (AAC)	15a,b	16	7	14	
12	U, 4C, 2 (AC), (AU), (AG), (AAC)					
13	U, 3C, G, 2 (AC), (A_4N)					
15	4U, 4C, G, (AC), (AU), (AAU)					
18	5U, 3C, G, (AU), (AAU)		22			
C	G, (AC), (AU), (AAU), (AAAN)	C	C	C	C	C

[a] Oligonucleotides numbered as in Figs. 2 and 3 were eluted from fingerprints shown in these Figs. and from fingerprints of poly(A)-tagged RNA fragments (not shown) prepared to derive the oligonucleotide map shown in Fig. 3 as described [14]. RNase A-resistant fragments of T_1-oligonucleotides were determined following published procedures [8, 14] except that digestion with RNase A was in 10 µl at 0,4 mg RNase A per ml for 3 hr.

[b] The columns marked on the right indicate 28S MH2, MH2AV-A&C, MCAV-A, PR-B and RPV-oligonucleotides numbered as below and in refs. 8–11, 14, 15, with the same composition as the respecitve counterparts of MC29 RNA.

[c] Numbers as in Fig. 2A

A comparison of the oligonucleotides of 28S MC29 and MH2 RNAs indicates that the two RNAs share about 5 out of 12–15 large oligonucleotides (see Tables 1 and 2). Three of these common oligonucleotides are also shared with other avian tumor viruses. However two are only shared by MC29 and MH2, i.e., nos. 7b and 8b of MC29 and nos. 9 and 10 of MH2. Hence it is conceivable that these oligonucleotides are part of the functionally related, specific *onc* genes of these two viruses.

It has been argued that (defective) transforming viruses are generated by recombination of a helper virus with an unknown (defective) virus preexisting in the cell or with cellular genetic elements [16–18, 23, 24]. The helper viruses isolated from the original stocks of MC29 and MH2 would appear to be likely candidates for one parent of such recombinational events. It would then be expected that the defective recombinant virus shares more sequences with its progenitor than with other possible helper viruses. To test this, the RNA of a cloned helper, isolated from the original MC29 stock, i.e.,

Table 2. T_1-oligonucleotides of 28S MH2 RNA[a]

28S MH2	RNase A digestion products	MC29[c]	RAV-7[c]	MH2A-A&C
Spot No.[b]				
1	U, 2C, G, 5 (AU), (AAU), (AAAC)			
(2)[b]	9U, 7C, 2 (AC), 2 (AU), (AG), (AAC), (AAU)		1	4
3	4U, 5C, G, 3 (AU), 2 (AAU), (AAAU)			
4a,b	3U, 9C, 2G, 4 (AC), 3 (AU), 3 (AAC), (AAAC)	1[d]		
(5)	6U, 9C, 2 (AC), (AU), (AAC), (AAG)		2	2
6a,b	9U, 16C, 2G, 2 (AC), 2 (AU), 2 (AAC), (AAAC), (A_5N)			
(7)	5U, 5C, G, 2 (AC), 3 (AU)		4	6
(8)	4U, 5C, (AC), (AAU), (AAAC), (AAAG)		5	
9	3U, 10C, (AC), (AAG)	7b		
10	2U, 3C, (AC), (AG), (AAU), (AAAC)	8b		
11a,b,c	10U, 15C, G, 3 (AC), 5 (AU), (AG), (AAG), (AAAG), (AnN)	9?		
12	2U, 4C, 2 (AC), (AU), (AG)			
13	U, 3C, 2 (AC), (AAG), (AAAU)			
(14)	4C, 2 (AC), (AU), (A_4G)		15	
15a,b	2U, 4C, G, 2 (AC), (AU), (AG), (AAC), (AnN)	11?		
(17)	2U, 4C, G, 2 (AC), (AU)		11	
(19)	10U, 7C, G, (AU)		8	
(20)	6U, 2C, G, 3 (AU), (AAU)		7	
cap[e]	class IV	cap		cap

[a] Oligonucleotides were eluted from fingerprints shown in Fig. 2 and their RNase A-resistant fragments were determined as described previously [14] and for Table 1. Some, but not all oligonucleotide numbers are the same as those used previously for more preliminary analyses of MH2 RNA [9, 10]. Due to scarcity of MH2 [^{32}P] RNA, some numbers are semi-quantitative, combining scintillation counting of RNase A-resistant fragments and reflecting visual estimates from autoradiograms.

[b] Numbers as in Fig. 2E and F. Oligonucleotides with homologous counterparts in RAV-7 or MH2AV-A&C helper virus RNAs are parenthesized (see text).

[c] Number denotations of homologous oligonucleotides found in MC29, RAV-7 and MH2AV-A&C RNAs (see Tables 3 and 4)

[d] 4a is MC29 no 1, see ref 40.

[e] see ref 22 for composition

MCAV-A, was fingerprinted (Fig. 2D) and the RNase-A resistant fragments of large oligonucleotides were compared to those of 28S MC29 RNA isolated from MC29 (RPV). It can be seen in Tables 1 and 3 and Fig. 2 that the two RNAs share only about three of their large T_1-oligonucleotides. In addition both RNAs as well as RPV RNA have the same 5′ terminal cap-oligonucleotide (marked in Fig. 1) [22]. The same was true for the relationship of 28S MH2 RNA to MH2AV-A and C RNAs, two helper viruses isolated from the original stock of the virus. These helper viruses did not share more oligonucleotides with MH2 RNA than with RAV-7 (compare Fig. 2 and Tables 2, 4 and 5). We conclude on the basis of oligonucleotide homologies, that 28S MC29 and MH2 RNAs are not more closely related to their original helper viruses than to the other helper viruses tested.

Table 3. Composition of T_1-oligonucleotides of MCAV-A RNA[a]

MCAV-A	RNase A digestion products	PR-B[c]
Spot No.[b]		
1	7U, 5C, (AC), (AU), (AG), (AAC), (AAU)	
2	6U, 10C, 2 (AC), (AU), (AAC), (AAG)	4
3a	4U, 7C, G, (AC), (AU), (AAAC)	6
3b	3U, 5C, (AC), (AAU), (AAAC), (A_4G)	
4	6U, 6C, G, 3 (AC), 3 (AU)	
5a,b	8U, 9C, G, 2 (AC), (AU), (AAG), (A_4C)	8
6	3U, 6C, G, 3 (AC), 2 (AU)	
7	2U, 5C, G, 2 (AC), (AU), (AAC)	14
8	4C, 2 (AC), (AU), (A_4G)	
10	U, 5C, 3 (AC), (AAG)	18
11	3C, (AU), (AG), (A_5N)	20b
13	6U, 2C, G, 3 (AU), (AAU)	
15	8U, 5C, G, (AU)	
18	5C, G, 4 (AC)	
21	5U, G, (AC), 2 (AU)	
22	5U, 3C, G, (AU), (AAU)	18
23	3U, 2C, G, 2 (AU), (AAU)	
24	5C, <2 (AC), (AG)	

[a] As in Table 1.
[b] As in Fig. 2D.
[c] Oligonucleotides of the same composition were found previously in PR-B (14) and MC29 (see Table 1)

Table 4. Composition of T_1-oligonucleotides of MH2AV-A and -C RNA[a]

MH2AV-A and C	RNase A digestion products	PR-B[c]
Spot no.[b]		Spot no.
1	7U, 10C, G, 3 (AC), (AAC)	
2	5U, 10C, 2 (AC), (AU), (AAC), (AAG)	4
3	6U, 10C, G, (AC), (AU), (AAAC)	6
4	8U, 6C, 2 (AC), 2 (AU), (AG), (AAC), (AAU)	
5	3U, 6C, G, 2 (AC), (AU)	
6	6U, 6C, G, 2 (AC), 4 (AU)	
7a,b	4U, 3C, 2 (AC), 2 (AU), (AG), (AAU), (AAAN), (A_4N)	
8	6U, 6C, (AAG), (A_5N)	
9	6U, 9C, G, (AAC)	
10	8U, 3C, 4 (AU), (AG), (AAU)	
11	6U, 2C, G, 4 (AU), (AAC)	
12	U, 2C, G, (AC), 2 (AAAN)	
13	4U, 3C, G, 2 (AU), (AAAN)	
14	5U, 4C, C, (AC), (AAAC), (AAAG)	
15	4U, 4C, (AC), (AAAC), (A_4G)	
16	2U, 5C, G, 2 (AC), (AU), (AAC)	14

[a] Determined as in Table 1.
[b] Numbers refer to oligonucleotides spots shown in Figure 2H.
[c] Oligonucleotides of the same composition were found in PR-B (14)

Table 5. T_1-oligonucleotides of RAV-7 RNA

Spot no.	RNase A digestion products[a]
1	7U. 4C. 2 (AC). 2 (AU). (AG). (AAC). (AAU)
2	4U. 7C. 2 (AC). (AU). (AAC). (AAG)
3	6U. 6C. G. (AC). (AU). (AAAC)
4	5U. 5C. G. 2 (AC). 3 (AU)
5	4U. 5C. (AC). (AAU). (AAAC). (AnN)
6	6U. 6C. 3 (AC). (AU). (AAG). (AAAN)
7	6U. 2C. G. 3 (AU). (AAU)
8	10U. 7C. G. (AU)
9	3U. G. (AC). 2 (AU)
10	4U. 1C. (AC). (AU). (AnG)
11	2U. 4C. G. 2 (AC). (AU)
12	2U. 4C. G. 2 (AAU). (AAAN)
13	2U. 4C. G. 2 (AC). (AU). (AAC)
14a,b	3U. 5C. 3 (AC). (AU). AG). (AAC). (AAAN)
15	4C. 2 (AC). (AU). (AnG)
16	2C. 2 (AC). (AAG)
17	2C. (AU). (AG). (AnN)
18	4U. 2C. G. (AU). (AAU)
19	2U. C. G. 3 (AU). (AAU)

[a] Oligonucleotides were prepared as for Tables 1–4. RAV-7 RNA was isolated from MH2 (RAV-7) virus as described in Fig. 1 and in the text. Quantitation of RNase A-resistant fragments was based on visual estimates of autoradiographs

The Relationship of 28S MC29 and MH2 RNAs to Each Other and to the RNAs of Other Avian Tumor Viruses Measured by Hybridization

To determine whether 28S MC29 and 28S MH2 RNA contain *src*-specific nucleotide sequences of avian sarcoma viruses and to investigate their relationship to each other and to the RNAs of helper-independent avian RNA tumor viruses, the RNAs were hybridized to various cDNAs. All hybridizations were carried out with an excess of cDNA and at increasing cDNA to RNA ratios to reach plateau values of maximal hybridization. Under our conditions, maximal hybridization of 28S MC29 RNA with homologous MC29 (RPV) cDNA and of Prague RSV-B (PR-B) RNA with homologous cDNA was about 93% (Table 6). To determine whether 28S MC29 and MH2 RNAs contain *src*-specific sequences each RNA was first hybridized to cDNA from PR-B which contains *src* and then to cDNA from transformation-defective (td) PR-B which lacks *src* [13–15]. It is seen in Table 6 that approximately the same percentage (62 to 66%) of each RNA was hybridized by each cDNA. The MC29 and MH2 RNA sequences hybridized by PR-B and td PR-B cDNA were not additive, because a mixture of these two cDNAs did not hybridize more than each by itself (Table 6). The PR-B cDNA used was shown to include *src*-specific sequences, because it was able to hybridize 13% more PR-B RNA than td PR-B cDNA (Table 6). This was the expected difference, because the *src* gene corresponds to about 13% of the

viral RNA [13]. It follows that MC29 RNA and MH2 RNA lack *src*-specific sequences. About 62 to 66% of MC29 and MH2 RNAs are related to the RNAs of PR-B, td PR-B and other independently replicating avian tumor viruses. Therefore these sequences are termed group-specific. At least 31% (i.e.. 93 minus 62) or 1700 nucleotides of each RNA would appear to be specific for MC29 or MH2, respectively. This is considered a minimal estimate because each electrophoretically prepared 28S RNA was contaminated with degraded helper virus RNA (c.f., Figs. 1 and 2). Helper virus RNAs including RPV RNA, 34S MH2AV-A and C RNA were 60 to 80% homologous to the RNAs of PR-B, td PR-B and of other avian tumor viruses (Table 6).

To test whether the specific sequences of MC29 and MH2 RNA (defined as those which did not hybridize with PR-B or td PR-B cDNA) are related to each other, 28S MH2 RNA was hybridized to MC29 (RPV) cDNA. About 64% of the RNA was hybridized (Table 6). If annealed with MC29 (RPV) and PR-B cDNAs, about 70% of 28S MH2 was hybridized. It follows that approximately 30% of the 28S MH2 RNA is unrelated to MC29 and PR-B RNA. The result that about 5–10% more 28S MH2 RNA was hybridized by MC29 (RPV) and PR-B cDNAs together than by each cDNA alone, may indicate that each of these cDNAs contains sequences related to different sequences of MH2. It is conceivable that PR-B cDNA would hybridize with segments of the above defined group-specific sequences of MH2 not represented in MC29 (RPV) cDNA and MC29 cDNA with segments of MH2-specific RNA not represented by PR-B cDNA. A specific relationship between MC29 and MH2 is consistent with the finding that MH2 and MC29 RNAs share two oligonucleotides not found in any other avian tumor virus tested here (see above).

To test further the notion mentioned above that defective transforming viruses are recombinants of a helper virus and an unknown parent, sequence homologies between the RNAs of MC29 and of its original helper virus, MCAV-A as well of MH2 and of MH2AV-A and C were determined. It is seen in Table 6 that 28S MC29 RNA is hybridized by MCAV-A cDNA to approximately the same extent, i.e., 61%, as by cDNA prepared from other avian tumor viruses. The same was true for 28S MH2 RNA and MH2AV-A and C cDNA (Table 6). The MCAV-A cDNA as well as the MH2AV-A and -C cDNA used, hybridized 94–96% of their homologous viral RNAs (Table 6). It is concluded that MC29 and MH2 are not more closely related to their original helper viruses than to other avian tumor viruses tested. This conclusion is consistent with the results described above that the T_1-oligonucleotides of MC29 and MCAV-A as well as those of MH2 and MH2AV-A and C RNAs are not more closely related to each other than to those of other avian tumor virus RNAs.

Mapping MC29-Specific and Group-Specific Sequences of MC29 RNA

To locate MC29-specific and group-specific sequences on MC29 RNA the following strategy was used: First the RNase T_1-resistant oligonucleotides of

Table 6. Hybridizations[a] of viral RNAs with viral cDNAs[b]

RNA	cDNA	% Hybridization at various cDNA/RNA ratios mean ± S.D. 5:1	20:1	50:1	100:1	150:1
28S MC29[c]	MC29 (RPV)	65 ± 6	84 ± 4	88 ± 1	93 ± 7	96
	RPV	46 ± 3	61 ± 0	66 ± 3	69 ± 4	
	PR-B	40 ± 2	54 ± 7	61 ± 8	61 ± 8	
	tdPR-B	50 ± 2	55 ± 6	61 ± 6	62 ± 7	
	PR-B + tdPR-B[h]	50	62 ± 6	60	61	
	MCAV-A	55	60 ± 3	61		
	PR-B + MCAV-A[h]	61	60 ± 5			
34S RPV	MCAV-A	64	65			
	MC29 (RPV)	48	81	80	80	
	tdPR-B	35	58			
34S MCAV-A	MCAV-A	91 ± 6	94 ± 5			
	MC29 (RPV)		49	80	80	
	RPV			75		
	PR-B		66	70		
28S MH2[d]	PR-B			60,5 ± 1	64	66,5 ± 5
	tdPR-B			58 ± 5	59,5	65,5 ± 2
	PR-B + tdPR-B[h]			62 ± 3		
	MC29 (RPV)			61,5 ± 5	61,5	64 ± 1
	MC29 (RPV) + PR-B[h]			67 ± 3		70,5 ± 6
MH2[e]	MH2AV-A and C			58 ± 5	60 ± 2	
34S MH2AV[d]	PR-B			79,5 ± 8		
	tdPR-B			79 ± 8	78	
	PR-B + tdPR-B[h]			86,5 ± 2		
	MC29 (RPV)			70 ± 5	75	
	MC29 (RPV) + PR-B[h]			84,5 ± 4		
MH2AV-A and C[f]	MH2AV-A and C			75 ± 4	96	
PR-B[g]	PR-B		93 ± 0	93 ± 1		
	tdPR-B		81	81		
	PR-B		94 ± 0	92 ± 1		
	tdPR-B		79	81		
RAV-7[e]	MH2AV-A and C				83	

[a] Each reaction mixture contained about 1 ng of ^{32}P RNA (2000 cpm/ng) and 5–100 ng of ^{3}H cDNA (50 cpm/ng). Hybridizations were in 4–5 µl of 70% deionized formamide/0,3 M NaCl/0,03 M Na citrate/15 mM Na phosphate, pH 7,0 and 0,05% $NaDodSO_4$ at 40° for 12 hr. Percentage nuclease-resistance is expressed as the radioactivity recovered in aliquots digested with nuclease relative to that found in undigested aliquots. Each value is the mean of 2 or 3 experiments using, in some cases, independent preparations of RNA and cDNA. Digestion was with RNases A (5 µg/ml), T_1 (10 units/ml) and T_2 (10 units/ml) for 30 min at 40° in 0,3 M NaCl/0,03 M Na citrate, pH 7,0. The background of nuclease-resistance of an aliquot heated at 100° in 0,01 M Na^+ was $<0,5\%$. Many of these data are from refs. 8, 9, 10. Ratios of cDNA to RNA represent maximal values for a given RNA sequence because cDNAs are nonequimolar mixtures of viral sequences and include viral double-stranded DNA as well as some cellular DNA species.

[b] cDNA was prepared as described previously [8, 9].

[c] Prepared by gel electrophoresis from MC29 (RPV) [8] as shown for Fig. 1.

the MC29-specific and of the group-specific segments of MC29 RNA were determined. MC29-specific RNA segments were recovered from RNA-DNA hybrids formed between viral RNA and MC29-specific cDNA. Group-specific segments of MC29 RNA were obtained from hybrids formed with cDNAs from other avian tumor viruses [10, 11, 26]. The location of the RNA segment to which a given oligonucleotide belonged was then deduced from a map of all large oligonucleotides of MC29 RNA. An oligonucleotide map describes the location of each large oligonucleotide relative to the 3′ poly(A)-coordinate of viral RNA. The location of a given oligonucleotide is deduced from the size of the smallest poly(A)-tagged RNA fragment from which the oligonucleotide can be obtained [14].

MC29-specific cDNA was prepared by hybridizing MC29 (RPV) cDNA to an excess of unlabeled RNA of RPV and of PR-B under conditions of moderate stringency (Fig. 3). In this way all but the MC29-specific sequences of this cDNA were converted to heteroduplexes leaving only MC29-specific cDNA single-stranded. This cDNA was then hybridized in a second step with 50–70S MC29 (RPV) [^{32}P] RNA essentially under the conditions described above. However incubation was for a shorter time, to minimize displacement of unlabeled RNA from heteroduplexes present in our preparation of MC29-specific cDNA by related or identical sequences of MC29 [^{32}P] RNA. After digestion of unhybridized MC29 (RPV) [^{32}P] RNA with RNases A and T_1, the resulting hybrids were prepared by chromatography on Biogel P100. Subsequently the hybrid was heat-dissociated and the [^{32}P] RNA was digested with RNase T_1. The resulting MC29-specific T_1-oligonucleotides were detected by fingerprint analysis (Fig. 3B) and their RNase A-resistant fragments were determined (Table 1).

The remaining oligonucleotides of MC29 RNA (Fig. 3A) are expected to derive from group-specific RNA segments, sequence-related to the RNAs of other members of the avian tumor virus group. Group-specific RNA sequences were identified as follows: 28S MC29 [^{32}P] RNA was prepared electrophoretically from 50–70S MC29 (RPV) RNA [8]. The RNA was then hybridized to an excess of PR-B and RPV cDNA as above (Fig. 3C). After hybridization the reaction mixture was treated with RNase T_1 to degrade unhybridized RNA. RNase A was not used for this purpose in order to preserve small MC29-specific oligonucleotide segments which are part of larger group-specific polynucleotide segments of MC29 RNA hybridized with PR-B and RPV cDNAs. Thus mismatches involving oligonucleotide segments with fewer than two Gs would register as complete hybrids in our conditions. Such mismatches are expected because neither RPV nor PR-B are immediate predecessors of MC29 and because group-specific sequences of avian tumor virus RNAs defined by hybridization are known to differ if compared by

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[d] Prepared from MH2 (MH2AV-A and C) propagated in macrophages as for c.
[e] Prepared from MH2 (RAV-7) as for c.
[f] 60–70S RNA from cloned MH2AV-A and C was used.
[g] Shown electrophoretically to be free of tdPR-B RNA [8].
[h] Each cDNA was present at the DNA to RNA ratio indicated.

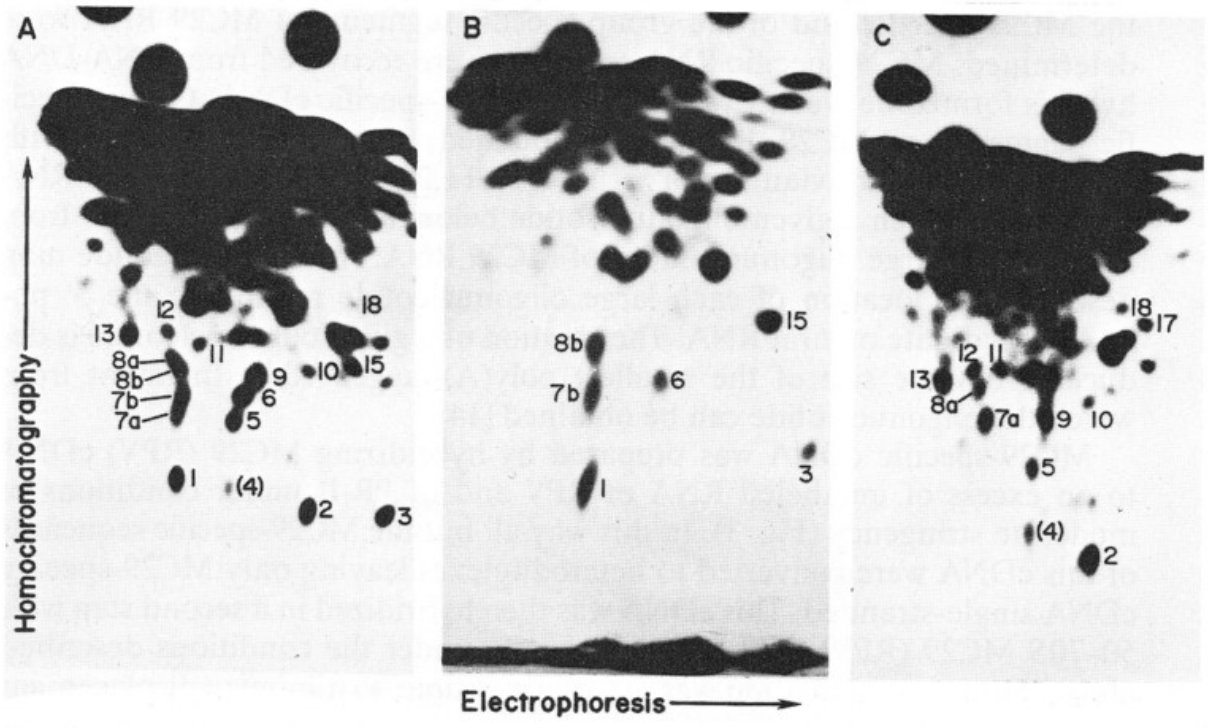

Fig. 3. RNase T_1-resistant oligonucleotides of whole 28S MC29 RNA (A). of MC29 RNA sequences that are not sequence-related to other avian tumor virus RNAs (MC29-specific) (B), and of MC29 RNA sequences that are sequence-related to other avian tumor virus RNAs (group-specific) (C). 28S MC29 [^{32}P] RNA was prepared electrophoretically from 50–70S MC29 (RPV) RNA, digested with RNase T_1 and subjected to 2-dimensional electrophoresis-homochromatography as described [8, 14]. Oligonucleotides were numbered as in Fig. 2 and previously [8–11]. In cases where double-spots were resolved, distinct oligonucleotides were denoted alphabetically (Fig. 2, Table 1). Oligonucleotide no. 4 is in parenthesis because it is thought to derive from contaminating RPV RNA [8] rather than from MC29 RNA (see Fig. 2). (B) To prepare MC29-specific sequences from MC29 RNA, 50–70S MC29 (RPV) [^{32}P] RNA was hybridized to MC29-specific cDNA. MC29-specific cDNA was made by incubating 2 μg MC29 (RPV) cDNA [8, 19] with 15 μg RPV RNA and 12 μg PR-B RNA for 12 hr at 40°C in 10 μl 50% formamide containing 0,45 M NaCl, 0.045 M Na citrate and 0,01 M Na-PO_4 pH 7.0. Subsequently, 1,5 μg of MC29 (RPV) [^{32}P] RNA (5×10^6 cpm/μg) was added in 20 μl of the above formamide buffer and incubation was continued for 1 hr. After digestion for 30 min at 40°C in 200 μl of 0,3 M NaCl, 0,03 M Na citrate containing 5 μg/ml RNase A and 50 units/ml RNase T_1, the resistant hybrid was isolated from the void volume of a Biogel P100 column ($12 \times 0{,}6$ cm) equilibrated in 0,1 M NaCl, 0,01 M Tris pH 7,4, 1 mM EDTA and 0,2% Na dodecylsulfate. Hybrid was extracted 3 times with phenol in the presence of 30 μg carrier yeast tRNA, then ethanol-precipitated, heat-dissociated in buffer of low ionic strength, digested with RNase T_1 and subjected to fingerprint analysis as above. The oligonucleotides from MC29-specific RNA segments so identified are underlined in the oligonucleotide map shown in Fig. 4. (C) To prepare avian tumor virus group-specific sequences of MC29 RNA, 0,25 μg of electrophoretically prepared 28S MC29 [^{32}P] RNA (Fig. 1, refs. 8, 9, 10) (2×10^6 cpm/μg) was hybridized with 1 μg of PR-B and 1 μg of RPV cDNAs for 12 hr in 25 μl of 70% formamide, 0,3 M NaCl, 0,03 M Na citrate, 0,02% Na dodecylsulfate and 0,015 M Na-PO_4 pH 7,0. The reaction product was heated to 50°C for 1 min in 0,15 M NaCl, 0,015 Na citrate pH 7,0 and treated with RNase T_1 (but not with RNase A) and otherwise as described for (B)

fingerprinting T_1-oligonucleotides [14, 15, 27, 28]. This is because fingerprinting detects specific oligonucleotides even in RNA sequences which differ by only a few percent of their nucleotides and which are closely related if compared by RNA-cDNA hybridization. The T_1-oligonucleotides of the result-

ing hybrid are shown in Fig. 3C and Table 1. They represent RNA sequences of MC29 RNA that are closely related to but not identical with sequences of PR-B and RPV RNA. It can be seen in Figs. 3 and 4 that the T_1-oligonucleotides of MC29 RNA fall into two non-overlapping sets, those representing MC29-specific and those representing group-specific sequences of MC29 RNA.

An oligonucleotide map of 28S MC29 [^{32}P] RNA was derived by fingerprinting poly(A)-tagged fragments of electrophoretically purified 28S MC29 RNA (Fig. 4). The approximate distance of each large oligonucleotide, numbered as in Figs. 2 and 3, is given in nucleotides as well as in relative map units, each originating at the 3′ poly (A) coordinate. The specific oligonucleotides (underlined in Fig. 4) mapped together between 0,4 and 0,7 units with some uncertainty about the relative order of nos. 2 and 8b. Oligonucleotides of group-specific sequences (not underlined) are found at the 5′ end and in the 3′ half of viral RNA. The group-specific sequences of the 5′ end included oligonucleotide no. 9, previously identified as a conserved element of the *gag* gene of other avian tumor viruses (see above) [27,28]. The 3′ half contained oligonucleotide no. 11, found previously at the *src* border of *env* in other virus RNAs [14,15,27,28] and the highly conserved C oligonucleotide which maps near the 3′ end of avian tumor virus RNAs [14].

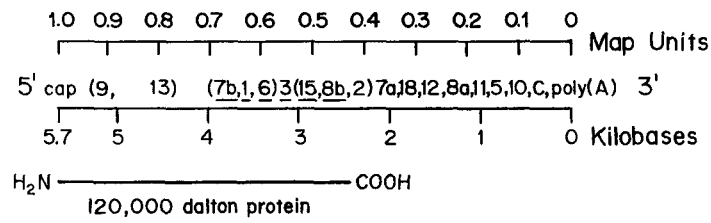

Fig. 4. Oligonucleotide map of 28S MC29 RNA. 28S MC29 [^{32}P] RNA (approx. 6×10^6 cpm) was prepared electrophoretically from 50–70S MC29 (RPV) RNA. The RNA was degraded by incubating 3 equal aliquots for 3, 6 and 9 min, respectively, in 0,05 M Na_2CO_3 at pH 11,0 and 50°C. Fragments were combined and poly(A)-tagged species selected on oligo(dT)-cellulose and fractionated into different size classes as described [14]. The T_1-oligonucleotides of 6 size classes of RNA fragments differing by approximately 1000 nucleotides from each other, were fingerprinted (not shown). Oligonucleotides of fragments were identified by their chromatographic properties and by their RNase A-resistant fragments (Table 1), and are numbered as in Fig. 2, 3 and Table 1. The resulting order of oligonucleotides is plotted on 2 scales, one denoting the approximate distance of an oligonucleotide from the 3′ poly(A)-coordinate in kilobases, the other denoting it in relative map units. When the relative order of oligonucleotides was uncertain, they are shown in brackets. Oligonucleotides from strain-specific sequences of MC29 RNA (Fig. 1B) are underlined and those from group-sequences (Fig. 1C) are not underlined. The bottom line represents the MC29-specific, 120000 dalton protein P120^{mc}. It is drawn in a position that is colinear with the RNA segment from which it was probably translated based on data described in the text and previously [10,11]

In Vitro Translation of MC29 RNA

To identify the products of the MC29 RNA genome, the RNA was translated in a cell-free system. Using this technique it has been possible to identify the *gag*, *pol*, *src*, and possibly also the *env* gene products of RSV [21,29,30,31].

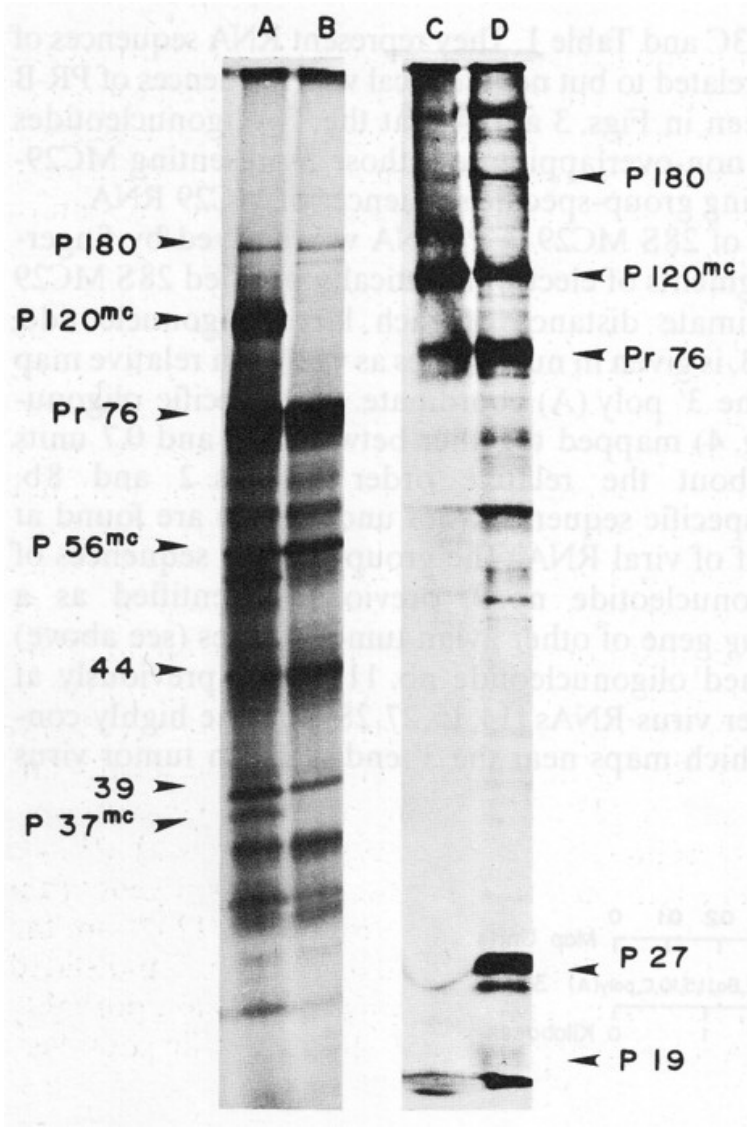

Fig. 5. Electrophoretic analysis of in vitro translation products of viral RNAs. RNAs were translated in the messenger-dependent rabbit reticulocyte lysate. [^{35}S]-methionine was present at 400 μCi/ml (600–1200 Ci/mmol). Products were analyzed by electrophoresis on a 12.5% polyacrylamide slab gel. The gel was autoradiographed after fixing and drying [4, 10, 11]. Tracks A and B show the products of total heat-denatured, poly(A)-selected (14) 50–70S virion RNAs from MC29 (RPV) and RPV (Track A), and from RPV alone (Track B). Track C shows the cell-free translation-products of MC29 (RPV) and RPV RNA (as in Track A), and Track D shows the proteins precipitated with antibody to disrupted avian myeloblastosis virus (AMV) (which contains mainly antibody to *gag* protein) from MC29 (RPV) infected cells. Immunoprecipitations were carried out as described [4, 20]. The viral proteins precipitated by anti-AMV serum were not precipitated by control serum, and P120^{mc} was not synthesized in RPV-infected cells (not shown)

MC29 (RPV) 50–70S RNAs were heat-denatured, poly (A)-selected, and translated in the messenger-dependent rabbit reticulocyte lysate [10, 11]. Products were analyzed by polyacrylamide gel electrophoresis. To identify the products specified by the MC29 RNA, the products of total poly (A)-selected MC29 (RPV) RNAs (Fig. 5, track A) were compared with those of RPV RNA alone (Fig. 5, track B). A number of products were in common between the two tracks, and these were assumed to be products of the RPV RNA.

These include a 180000 dalton-molecular weight polypeptide (P180), which is believed to be the *gag-pol* gene product [31]; Pr76, the primary product of the *gag* gene [30,31,32] and a number of smaller products, most of which are immunoprecipitated by antiserum to the *gag* protein, P27, and were only synthesized from full-length 34S RNA, suggesting that they are premature termination products of the *gag* gene [11].

In addition to these products there were three polypeptides which are specific to MC29 RNA. They have molecular weights of 120000, 56000, and 37000 daltons on this gel system, and will be denoted as $P120^{mc}$, $P56^{mc}$, and $P37^{mc}$ (Fig. 5). $P120^{mc}$ was a major product of the MC29 (RPV) RNA mixture, and was synthesized with the same order of efficiency as Pr76 (since they have a similar number of methionine residues [11]). A protein of similar size was recently found in MC29-infected cells and was shown to contain serological determinants of the viral *gag* gene proteins [4]. To test the relationship of the two proteins, $P120^{mc}$ was compared electrophoretically to its presumed counterpart precipitated from MC29-infected cells with antibody to disrupted avian myeloblastosis virus (AMV), which includes antibody to *gag* proteins. It can be seen that both proteins were electrophoretically identical (Fig. 5C, D). In addition $P120^{mc}$ synthesized in vitro was specifically immunoprecipitated by antiserum to P27 of AMV, the major *gag* gene protein, indicating that it contains determinants of *gag* proteins (not shown). $P120^{mc}$ was not recognized by antisera against products of the *pol* and *env* genes (not shown). Further evidence that the in vivo and in vitro-made $P120^{mc}$ are the same has been obtained recently [11]. We conclude that the $P120^{mc}$ translated in vitro from viral RNA and that found in MC29-infected cells are probably the same and that $P120^{mc}$ contains *gag*-related and MC29-specific peptides.

Discussion

The RNA and Gene Products of MC29 and MH2

The finding that different pseudotypes of MC29 or MH2 contained physically and chemically very similar or identical 28S RNAs, but 34S RNAs that varied with the respective helper virus, proved that the 28S RNAs are MC29- or MH2-specific. Each 28S RNA contained 30–40% of specific nucleotide sequences, which only hybridized with homologous cDNAs and 60–70% of sequences which hybridized with cDNAs of other avian tumor viruses which were termed group-specific. *Src* gene-related sequences were not detected in 28S MC29 or MH2 RNA.

In the case of 28S MC29 RNA, the specific sequences, identified by the large T_1-oligonucleotides they contain, mapped about 0,4 to 0,7 map units from the poly (A) end of the RNA (Fig. 4). The observation that MC29 and MH2 share two specific oligonucleotides, which in MC29 RNA mapped in a contiguous, MC29-specific RNA segment (Fig. 4), suggests that the specific sequences of the two viruses are related. This relationship has since been extended to three oligonucleotides [see Table 2 and ref 40].

In vitro translation of MC29 RNA generated one major 120000 dalton protein product, and two minor proteins of 56000 and 37000 daltons. The 120000 MC29 protein included protein sequences serologically related to the *gag* gene of other avian tumor viruses. Since one *gag* gene-related oligonucleotide, i.e., no. 9, was found near the 5′ end of MC29 RNA and since the *gag* gene of other avian tumor viruses maps near the 5′ end of their RNAs [27,28] it appears plausible that the *gag* gene-related portion of the 120000 MC29 protein was translated from the 5′ end of the RNA and that the remainder was translated from the MC29-specific sequences of the viral RNA (Fig. 4). Our recent observation that only full-length 28S MC29 RNA can be translated into $P120^{mc}$ [11] also argues for a 5′ map location of this protein, because eukaryotic mRNAs only effectively use one initiation site near the 5′ terminus [33,34,35].

The group-specific sequences that MC29, MH2 and other avian tumor viruses have in common are nearly indistinguishable, if compared by hybridization, but are distinct in each viral RNA if analyzed by the more sensitive method of fingerprinting, which detects single base changes. Since MH2 and MC29 do not express replicative genes, we can only speculate on the function of the group-specific sequences of their RNAs. Some of these sequences must play direct roles in virus replication by providing specific sites for packaging of viral RNA into helper virus proteins, for reverse transcription of viral RNA and for dimer linkage of 28S RNA monomers [6,8,9,10]. The *gag*-related, group-specific sequences of MC29 are translated into $P120^{mc}$ and may as such be involved in transformation (see below). Further analyses of $P56^{mc}$ and $P37^{mc}$ are necessary to determine whether their sequences overlap with $P120^{mc}$ or with each other or whether they correspond to distinct segments of viral RNA, since MC29 RNA may code for approximately 200000 daltons of protein.

What is the Onc Gene of MC29 and MH2?

Since neither MC29 nor MH2 contain *src* gene-related sequences, there are two different hypotheses as to which RNA sequences of these viruses represent their *onc* genes. One suggests that the specific sequences constitute the *onc* gene, while an alternative hypothesis suggests that their defective replicative genes i.e., group-specific sequences function as transforming genes. We prefer the first hypothesis for several reasons.

The idea that specific RNA sequences apparently unrelated to the replicative genes might be specific *onc* genes is proven for RSV [13,14,15,19] and has also been postulated for defective murine sarcoma [16,17,18,23,24] and acute leukemia viruses [9,10,36]. The existence of specific sequences in MC29 and MH2, which are related to each other, but unrelated to the other avian tumor virus RNAs tested, suggests that these sequences may belong to a family of related genes, possibly the functionally related [1–5,9,10] *onc* genes of MC29 and MH2. A specific *onc* gene for this class of viruses also corresponds with the distinct transformed phenotypes of MC29- or MH2-infected fibroblasts, which differ from those of RSV-transformed cells

[1–5, 37]. The result that MC29-specific and group-specific sequences are translated into a specific protein, $P120^{mc}$, and that the same protein is also found in transformed cells [4] (and not in large quantity in the virion [unpublished]) suggests that this protein may be involved in cell transformation. A possibly analogous non-structural protein of 120000 daltons, that contains *gag*-related and specific peptides has been found in cells transformed by MH2 [5] and by the defective Abelson murine leukemia virus [38].

Further work correlating the specific RNA sequences of MC29 and MH2 with the specific determinants of their $P120^{mc}$ proteins is expected to support the hypothesis that this class of protein may be involved in transformation. Moreover, it is important to determine whether the 56000 and 37000 molecular weight MC29 proteins synthesized in vitro are also synthesized and functional in MC29-infected cells. While a definite answer to the question of whether the specific sequences of MC29 or MH2 and the $P120^{mc}$ proteins are involved in transformation can only be given if genetic variants become available, our data allow us to conclude that the *onc* genes and gene products in different prototypes of the avian tumor virus family, are different. Thus MC29 and MH2 must transform cells with gene products and possibly by mechanisms that differ from those of RSV.

Our data, that viruses with specific oncogenicity carry specific *onc* genes does not exclude roles for other viral genes, including those of the helper virus, in determining the oncogenic spectrum of a defective transforming virus. For example, the oncogenic spectrum of a defective virus should be greatly influenced by the *env* gene of its helper virus which provides the envelope glycoproteins for the defective virus. Since the cellular receptors for viral envelope glycoproteins differ greatly among different animals [39] and even among different target cells of the same animal [25] different helper viruses may deliver the same *onc* genes into specific target cells and thus cause a different form of cancer.

Acknowledgments

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