RNA and Protein Encoded by MH2 Virus: Evidence for Subgenomic Expression of *v-myc*

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MH2 and MC29 are highly related *myc*-containing avian retroviruses. We found that MH2, unlike MC29, synthesizes a 2.6-kilobase subgenomic mRNA containing *myc* sequences as well as sequences from the 5' end of the genome. A 57-kilodalton protein containing *myc*, but not *gag*, sequences ($p57^{myc}$) was detected by hybrid selection and in vitro translation of RNA from MH2-transformed cells. Gradient separation of MH2 intracellular RNAs indicated that $p57^{myc}$ is encoded by the subgenomic RNA. A highly oncogenic MH2 virus variant (MH2YS3) (M. Linial, *Virology* 119:382-391, 1982) was shown to encode only $p57^{myc}$ and not P100, the previously described MH2-encoded polypeptide (Hu et al., *Virology*, 89:162-178, 1978). Cells transformed by subclones of this virus synthesized predominantly the 2.6-kilobase RNA rather than genomic 5.4-kilobase RNA. These results suggest that only $p57^{myc}$ is required for maintenance of the transformed state after MH2 infection.

MH2 is a member of the MC29 group of avian acute leukemia virus. This family of viruses, which includes OK10 and CMII as well as MC29 and MH2, induces myelocytomatosis and liver and kidney carcinomas in vivo (9) and transforms fibroblasts and macrophages in vitro (3). The sequences responsible for the oncogenicity of these viruses are encoded by an oncogene, *v-myc*, which was probably derived from a host cell sequence, *c-myc* (8, 22, 25).

The genomes of MH2 and MC29 are highly related; however, the viruses exhibit some differences in transformation specificity (13). In particular, MH2 is much more oncogenic than MC29 when injected into young, immunologically competent quail. MH2 also exhibits 20-fold greater efficiency of transformation of quail macrophages in vitro relative to MC29. These biological differences between MH2 and MC29 may be due to differences in expression of the viral oncogenes. MC29 encodes a 110-kilodalton (K) polypeptide (P110^{*gag-myc*}), which contains structural protein (*gag*) and oncogenic sequences (*myc*) and is necessary for oncogenic transformation (4, 5, 19). MH2 encodes a 100K *gag*-containing protein (P100); however, there is no direct evidence as to whether this protein is required for transformation or whether it contains *myc* sequences (15; M. Hayman, personal communication). In this report, we show that unlike MC29, MH2-infected cells express a 2.6-kilobase (kb) subgenomic 5' *myc* mRNA in addition to the genomic RNA. This 2.6-kb RNA is shown, by hybrid-selected translation experi-

ments, to encode a 57K *myc* protein ($p57^{myc}$) which may be the MH2 transforming protein. RNA encoding $p57^{myc}$ was also detected in cells transformed by a variant of MH2, MH2YS3, in which no synthesis of P100 could be detected.

MATERIALS AND METHODS

Viruses, cells, and tissue culture. MH2P100+ (rescued with MCAV-A) was derived from MH2 nonproducer cells and was provided by P. Vogt, and MH2YS3 (MH2AV) was from this lab (13).

The transformed quail cell lines used were MC29 QNP (clone Q8) (4), provided by K. Bister; MH2P100+ QNP was from J. Neil and P. Vogt (12); and MH2YS3 producer cell lines were from this lab (13).

MH2YS3 viral clones were obtained by picking single foci under agar from infected quail embryo fibroblasts (clones 24, 26, and 28) or quail peripheral blood macrophages (clones 40 and 41). In both cases, plates used contained fewer than 10 foci (multiplicity of infection of 10^{-3} to 10^{-4}).

In vivo labeling of cells and immunoprecipitation. Methods for labeling cells with [³⁵S]methionine, for immunoprecipitation, and for gel electrophoresis (24) as well as the preparation of anti-RSV serum have been described (13).

Isolation of viral RNA. Viral particles from MH2P100+ or (MCAV)-MH2YS3-transformed cells were gradient purified and suspended in 100 mM NaCl-10 mM Tris (pH 7.4)-1 mM EDTA. RNA was extracted by adjusting the virus suspension to 0.5% in sodium dodecyl sulfate (SDS) followed by incubation at 37°C for 2 to 3 min. The RNA was then extracted twice with a mixture of phenol (buffer saturated)-chloroform-isoamyl alcohol (1:1:0.04). The aqueous

phase was adjusted to 0.2 M in sodium acetate and ethanol precipitated with 2 volumes of 100% ethanol. The ethanol precipitates were washed in cold 95% ethanol, air dried, and suspended in water.

Isolation of cellular RNA and Northern blot analysis. Total cellular RNA was prepared by a modification of the guanidine hydrochloride method (1, 27), and polyadenylated [poly(A)⁺] RNA was prepared as described (2) by passing total RNA once over a column of oligodeoxythymidylic acid-cellulose (Collaborative Research, Inc.). Poly(A)⁺ RNA was size fractionated on sucrose gradients. A 200-μg sample of poly(A)⁺ RNA was suspended in 10 mM Tris (pH 7.5)–1 mM EDTA (pH 8)–0.5% SDS, heated at 80°C for 2 min, and then stored on ice. The RNA was then layered on 12-ml 15 to 30% sucrose gradients containing 50 mM NaCl–10 mM Tris (pH 7.5)–1 mM EDTA (pH 8)–0.5% SDS. The gradients were centrifuged for 8 h at 38,000 rpm in an SW41 rotor at 25°C. Fractions were collected from the bottom of the gradient, and 20 μg of calf liver tRNA per ml was added as carrier; the fractions were then adjusted to 0.2 M in sodium acetate and precipitated with 2 volumes of ethanol. RNA size was estimated from the positions of 18S (2.0 kb) and 28S (5.2 kb) quail rRNAs.

For Northern blot analysis, total viral or cellular poly(A)⁺ RNA was denatured by glyoxal treatment (14). The RNA was then fractionated by electrophoresis on 1% agarose gels, transferred to nitrocellulose paper, and hybridized with nick-translated [³²P]DNA probes (21,28).

In vitro translation and p15 protease cleavage of in vitro translation products. Viral or cellular RNAs were translated in a reticulocyte cell-free system (17). RNA samples were translated in 12.5- or 25-μl reactions with 1 μCi of [³⁵S]methionine per μl (ca. 800 Ci/mmol; New England Nuclear Corp.) and incubated at 30°C for 2 h. Before the addition of sample buffer and electrophoresis on SDS-polyacrylamide gels, the translation reactions were incubated at 37°C for 20 min with 30 μg of RNase A per ml and 16 mM EDTA. For p15 protease cleavage, 12.5 μg of each reaction was incubated at 37°C for 60 min with 10 μg of avian myeloblastosis virus. The avian myeloblastosis virus virions, which contain p15 protease, had been disrupted by incubation at 37°C for 2 min in the presence of 1% Nonidet P-40 (30).

Hybrid-selected translation. The procedure used for hybrid selection (20) was modified, following suggestions of Jim Lewis (personal communication). The plasmid DNA used for preparation of filters (*c-myc*, *gag*, or *rep*) was linearized by restriction enzyme cleavage followed by phenol-chloroform extraction and ethanol precipitation. The DNA pellets were suspended in water, boiled for 1 min, and then cooled on ice. DNA filters were prepared by spotting 5 μg of linearized and denatured plasmid DNA on 6-mm nitrocellulose filter circles. The filters were air dried and then washed in 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) for 30 min and baked for 2 h at 80°C. The filters were prehybridized at 50°C in 0.1 ml of 65% formamide–400 mM NaCl–10 mM PIPES [piperazine-N,N'-bis(2-ethanesulfonic acid)] (pH 6.4)–4 mM EDTA (pH 8). After 1 to 2 h, the prehybridization solution was removed and replaced with 0.1 ml of the same mixture, which also contained poly(A)⁺ RNA. The hybridization reactions were incubated at 50°C for

2 to 3 h. The hybridization solution was then removed, and the filters were washed three times at room temperature in 1× SSC–0.5% SDS and five times in 0.1× SSC–0.1% SDS (wash buffer held at 60°C). The filters were then incubated at 60°C for 5 min in 2 mM EDTA and washed once in 2 mM EDTA (at room temperature) and once in water; 0.3 ml of water plus 10 μg of calf liver tRNA were then added to the filters, and the RNA was eluted by boiling for 1 min. The samples were quickly cooled on ice and the RNA solution was adjusted to 0.2 M in sodium acetate and precipitated with 2.5 volumes of ethanol. The RNA precipitate was pelleted, washed in 95% ethanol, dried, suspended in a small volume of water and added to the reticulocyte cell-free system.

Cloned DNAs used as probes in Northern blots and hybrid-selected translation. Sources and characterization of probes used were as follows: *c-myc* (m), *Sst*I fragment of the chicken cellular *myc* gene cloned in pSV2, a derivative of pBR322 (C. Pachl, et al., unpublished data) (7); *rep* (r), pSRA2, the total Rous sarcoma virus genome (SR-A) cloned into the *Sal*I site of pBR322 (obtained from J. M. Bishop); *gag* (g), 1.35-kb *Bam*HI *gag* gene-specific subclone of SR-A (obtained from J. M. Bishop); 5', a fragment of the viral long terminal repeat containing the U5 and R regions and about 50 bases of U3 (L fragment of reference 16).

RESULTS

***gag*-containing proteins synthesized by MH2P100+ and MH2YS3.** It has been shown previously that a variant of MH2 virus does not synthesize the MH2-encoded *gag*-containing polyprotein P100 originally described by Hu et al. (12), although this virus, MH2YS3, transforms macrophages and fibroblasts in culture and induces tumors in birds (13). As shown in Fig. 1, in vivo labeling of cellular proteins followed by immunoprecipitation with anti-*gag* antiserum revealed P100 in standard MH2 cells (called here MH2P100+), but not in MH2YS3-transformed quail embryo fibroblasts. However, the MH2YS3-transformed cells appeared to contain a mixture of viruses. We were able to clone viruses from these cells (an example is clone cl.28 in Fig. 1) which did encode P100. However, a majority of cultures infected and transformed by single viruses cloned from MH2YS3 (six of eight examined) lacked P100; an example was clone cl.44 (Fig. 1). In addition, the majority of cells cloned from MH2YS3 mass-infected fibroblast or macrophage cultures lacked P100; we detected none of this protein in 26 transformed-cell subclones examined. When MH2P100+ virion RNA was translated in vitro by using the rabbit reticulocyte lysate system (Fig. 1), a large amount of P100 was detected in addition to the major helper virus-encoded protein Pr76^{*gag*}. Although Pr76^{*gag*} was detected when MH2YS3 virion RNA was translated in vitro, no P100 was detected. This suggests that the inability to detect P100 in vivo in MH2YS3

FIG. 1. Proteins encoded by MH2 virions in vivo and in vitro. For in vitro translation of MH2 viral RNAs, viral RNA from MH2P100+ (rescued with MCAV-A) and MH2YS3 virions was translated in a reticulocyte cell-free system by using 0.4 μ g of RNA per 25- μ l reaction. These RNAs were a mixture of MH2- and helper virus-encoded sequences. The translation products of MH2P100+ and MH2YS3 viral RNA with (+) or without (-) p15 digestion were analyzed on 12.5% polyacrylamide gels followed by autoradiography. Endo, Endogenous polypeptides synthesized by the cell-free system in the absence of added RNA template. For in vivo labeling of cells transformed by MH2 and MC29, MC29 QNP MH2P100+ and MH2YS3-transformed producer cell lines (mass-infected quail embryo fibroblasts, and subclones cl.28 and cl.44) were labeled for 2 h with [35 S]methionine, lysed, and immunoprecipitated with anti-Rous sarcoma virus serum. The immunoprecipitates were analyzed in 12.5% polyacrylamide gels followed by autoradiography.

cells is not caused by a block in translation of viral RNA, but that the P100 template is lacking. A protein of about 130K (P130) was detected after in vitro translation of MH2YS3 virion RNA. This protein contained *gag* peptides since it was immunoprecipitated by anti-*gag* serum (data not shown) and it was also cleaved by the viral protease p15. This protease has been shown to be specific for *gag*-containing proteins and is capable of cleaving *gag* fusion proteins (29, 30). Since P130 is not detected intracellularly, its origin and function are unknown; it may represent an artifact of in vitro translation.

Translation of hybrid-selected RNA. Because of these results, which suggest that transformation by MH2 can occur in the absence of P100, it was of interest to determine whether MH2 encodes any other *myc* protein. Since anti-*myc* antiserum is not available, hybrid-selected translation experiments were performed to detect *myc* proteins. In these experiments, plasmid DNAs containing *c-myc* or viral structural genes

(*gag* or *rep*) were bound to nitrocellulose filters and hybridized to poly(A)⁺ mRNA from MC29- or MH2-transformed cells. The RNA selected by hybridization was eluted from the filters and translated in vitro. When RNA from MC29-transformed cells was hybrid selected with *myc* DNA, only the P110^{*gag-myc*} polypeptide was detected (Fig. 2). This protein was also detected after selection of MC29 RNA with *gag* DNA, as was the viral structural protein precursor Pr76^{*gag*} encoded by the helper virus RNA. These results agree with the finding of only genome-sized *myc* RNA in MC29-infected cells (Fig. 3) (26). RNA selected from MH2P100+ cells by either *gag* or *myc* DNA directed the translation of P100, showing that at least the RNA encoding this protein contains *myc* sequences. In addition, *myc* DNA selected an RNA encoding a protein of about 57K which was not selected by *gag* DNA (p57^{*myc*}). A 57K protein was also seen when MH2YS3 cellular RNA was selected by *myc* DNA. The *rep* probe used in this experiment did not hybrid select any RNA encoding a protein comigrating with p57. However, several protein bands were detected with similar sizes. It has been shown that in vivo labeling of MH2YS3 cells followed by immunoprecipitation by anti-RSV serum reveals a protein of about 57K (13). This in vivo-detected protein is cleaved by p15 protease and is presumably a fragment of *gag*. Fragments translat-

FIG. 2. Translation of hybrid-selected MC29 and MH2 poly(A)⁺ cellular RNAs. Poly(A)⁺ RNA from an MC29-transformed quail cell line (IQ1a, derived from an MC29-induced liver tumor [13]) and from MH2P100+ and MH2YS3-transformed producer cells was isolated and selected with *c-myc*, *gag*, or *rep* DNA. The selected RNAs were translated in the reticulocyte cell-free system and the translation products were analyzed on 12.5% polyacrylamide gels followed by fluorography. Each lane represents polypeptides synthesized in vitro from MC29 or MH2 cell RNAs hybrid selected by *myc*, *gag*, or *rep* (Rous sarcoma virus) DNA; endo, endogenous proteins synthesized in the cell-free system without added RNA.

ed after selection by the rep probe may be related to this *gag* protein observed in vivo and may represent the product of termination at the end of the *gag* sequences. As expected from the results in Fig. 1, P100 was not detected after hybrid selection and translation of the MH2YS3 cellular RNA. This suggests that MH2YS3 cells, as well as virions, do not contain the RNA template for P100, and that p57^{myc} may be the MH2-transforming protein.

Analysis of virus-specific RNAs in MH2-transformed cells and virions. To determine what RNA species might encode p57^{myc}, Northern blot analysis was performed with both viral and cellular poly(A)⁺ RNAs (Fig. 3). Virions released from MH2P100+ cells after superinfection with helper virus contained a 7.8-kb helper virus genomic RNA detected with DNA probes from non-*myc* regions of the genome (*gag*, 5', or rep), and a 5.4-kb MH2 genomic-length RNA detected by 5', *gag*, and *myc* sequences. Virions released from MH2YS3 cells contained a large excess of helper virus, as evidenced by the large amount of 7.8-kb RNA and a small amount of *myc*-containing RNA. Three faint *myc*-containing RNAs of 6.2, 5.4, and 5.0 kb were detected. The identity and relationship of these three RNAs await analysis of recloned MH2YS3 virions from the mixed population.

MC29 nonproducer cells contained the 5.4-kb MC29 genomic length RNA previously described (26) as well as a larger 6.2-kb RNA (Fig. 3). The relationship of this larger RNA to genomic RNA is currently unknown. No smaller RNAs could be detected with the *myc* DNA probe. In contrast, MH2P100+ cells (infected with helper virus) contained genomic length 5.4-kb RNA detected by *gag*, *myc*, and 5' probes, as well as an additional RNA of 2.6 kb detected with 5'- and *myc*-specific RNA probes, but not

FIG. 3. Northern blot analysis of RNA from MH2-transformed cells and virions. Viral and cellular poly(A)⁺ RNAs were denatured and electrophoresed on 1% agarose gels, transferred to nitrocellulose paper, and hybridized with nick-translated [³²P]DNA probes: m, c-*myc*; g, *gag*; 5', 5' region of long terminal repeat; r, pSRA2 (total Rous sarcoma virus genome).

FIG. 4. Northern blot analysis of RNA from cells transformed by MH2YS3 subclones. Cellular poly(A)⁺ RNAs were size fractionated as described in the legend to Fig. 3 and probed with nick-translated c-*myc* DNA.

with the *gag* probe. The poor hybridization of the 5' probe to the 2.6-kb RNA is caused by preferential loss of this RNA from the filter after removal of the preceding probes. In another blot (data not shown) the 5' probe hybridized at least as well to the 2.6-kb RNA as it did to the 5.4-kb RNA from MH2P100+ cells. We do not as yet know whether the 2.6-kb RNA contains *env* or other viral sequences to the 3' end of *myc*. The poor hybridization of 2.6-kb RNA to a representative probe containing Rous sarcoma virus *env* sequences is suggestive of a lack of *env* sequences in the RNA. Helper virus-related 7.8-kb genomic RNA was detected with the *gag* and 5' probes, and 3.5-kb *env* mRNA was detected with the 5' probe. The RNA pattern in MH2YS3 cells was more complex than that in MH2P100+ cells; four distinct *myc*-containing RNAs were seen, with sizes of 5.4, 4.5, 3.6, and 2.6 kb. MH2YS3 cells also contained helper virus 7.8-kb genome RNA and 3.5-kb *env* mRNA. A summary of the RNA data is presented in Table 1.

Since MH2YS3 cells appeared to contain a mixture of viruses, the virions were recloned and cellular RNAs analyzed. Three types of clones were isolated, the majority of which did not direct the synthesis of P100. Clone 26 and clone 28 (which does synthesize P100, Fig. 1) are examples of viruses which express a complex pattern of viral RNA in transformed cells (Fig. 4). These clones directed the synthesis of the

TABLE 1. Viral RNAs present in MH2-transformed cells and virions^a

RNA size	Virions				Cells									
	MH2P100+		MH2YS3		MH2P100+				MY2YS3				MC29NP	
	<i>myc</i>	<i>gag</i>	<i>myc</i>	<i>gag</i>	<i>myc</i>	<i>gag</i>	5'	rep	<i>myc</i>	<i>gag</i>	5'	rep	<i>myc</i>	<i>gag</i>
7.8 ^b	—	+	—	+	—	+	+	+	—	+	+	+		
6.2 ^c			+	+									+	+
5.4 ^c	+	+	+	+	+	+	+	+	+	+/-	+/-	+/-	+	+
5.0 ^c			+	+										
4.5 ^c									+	+	+	+		
3.6 ^c									+	—	—	—		
3.5 ^d									—	—	+	+		
2.6 ^c					+	—	+	+/-	+	—	+	+/-		

^a All RNAs detected in our Northern blot experiments (Fig. 3) are included. A blank denotes absence of that RNA size in the sample. DNA probe and RNA sources are as described in the legend to Fig. 3. Hybridization of an RNA to the rep probe would be indicative of either *gag* or *env* sequences since 5' sequences comprise only 1 to 2% of the probe. Poor hybridization of rep to the 2.6-kb *myc* fragment suggests, but does not prove, that it lacks *env* sequences.

^b Helper virus genome-length RNA.

^c RNAs containing *myc* sequences.

^d Helper virus *env* mRNA.

5.4-kb genomic and 2.6-kb subgenomic *myc* mRNAs as well as several different-sized *myc* RNAs of greater than 6 kb and about 4.5 to 5 kb (as summarized in Table 2). All of these RNAs also contain 5' sequences (data not shown). A second group of clones was detected, such as clone 24 (Fig. 4) which, similar to MH2P100+, contained the 5.4- and 2.6-kb 5'-*myc* RNAs as well as minor species. Two clones, 40 and 41, were also isolated which expressed a very simple pattern of *myc* RNAs in transformed cells, primarily the 2.6-kb subgenomic 5'-*myc* in RNA. Very little genome-sized RNA was detected and, as expected, the titer of virus produced from cells transformed by clones 40 and 41, coinfecting with helper virus, was low (Table 2). These results strongly suggest that the 2.6-kb subgenomic *myc* mRNA is sufficient for maintenance of viral transformation since all of the cells of clones 40 and 41, from which RNA was extracted, were transformed. We have not as yet tested the oncogenicity of clone 40 and 41 cells containing primarily the 2.6-kb RNA, nor that of the virus released from these cells.

Translation of hybrid-selected sucrose gradient fractionated MH2P100+ cell mRNA. Analysis of *myc* RNAs in cells transformed by MH2YS3 clones suggests that the 2.6-kb 5'-*myc* poly(A)⁺ RNA, which is also present in MH2P100+ cells, encodes the MH2-transforming protein. A likely candidate for this protein is p57^{myc}, described in Fig. 2. To substantiate this conclusion, poly(A)⁺ RNA from MH2P100+-transformed cells was size fractionated by sucrose gradient centrifugation and the RNA in each fraction was selected with both *myc* and *gag* DNA. *myc* DNA selected the 2.6-kb mRNA which encodes p57^{myc} as

well as the 5.4-kb genomic RNA which encodes P100 (Fig. 5). The several smaller peptides which were also synthesized from the 5.4-kb RNA may represent premature termination peptides of the in vitro translation system. As expected, the genomic 5.4-kb RNA encoding P100 was also selected by *gag* DNA, but no p57^{myc} was translated after selection by *gag*.

DISCUSSION

At least four independent isolates of avian retroviruses containing *myc* as their oncogene have been reported (10). The prototype virus MC29 encodes only a genome-sized RNA and a single *myc*-containing polypeptide P110^{*gag-myc*}. Two other isolates, CMII and OK10, contain non-helper-related sequences completely homologous to MC29 *myc* (18, 22). However, OK10, unlike MC29, has been shown to encode a 3.5- to 3.6-kb subgenomic *myc* mRNA as well as genomic length RNA (6, 23). The fourth virus, MH2, may contain sequences nonhomologous

TABLE 2. Properties of MH2YS3 viral subclones

Viral clone	Size of <i>myc</i> RNAs (kb)	Titer (focus-forming units/ml) ^a	P100
24	5.4, 4.5 ^b , 2.6, 1.5 ^b	1.6 × 10 ³	—
26	6.4, 5.4, 4.7, 4.3, 2.6	1 × 10 ³	—
28	6.2, 5.4, 4.3, 2.6	8 × 10 ³	+
40	5.4 ^b , 2.6, 1.5 ^b	2.3 × 10 ²	—
41	5.4 ^b , 2.6	3 × 10 ¹	—

^a Assayed on quail embryo fibroblasts.

^b Minor species (less than 10% of 2.6 kb).

FIG. 5. Translation of hybrid-selected sucrose gradient-fractionated cellular RNA from MH2P100⁺-transformed cells. Poly(A)⁺ RNA was size fractionated on 15 to 30% sucrose gradients. The RNA in each fraction was ethanol precipitated and suspended in 60 μ l of water; 30 μ l of each fraction was hybrid-selected with either *myc* or *gag* DNA. The hybridization conditions were as described in the text, except that the prehybridization and hybridization solutions contained 50% formamide–700 mM NaCl–10 mM PIPES (pH 6.4)–4 mM EDTA (pH 8) and hybridization was at 41°C for 18 h.

to MC29 (22, 25). All of these viruses transform several target cells in vitro, including fibroblasts and macrophages, and induce carcinomas and sarcomas in newborn birds.

In this report, we have shown that MH2 resembles OK10 in transcription of viral RNA, since the MH2 genome encodes a 2.6-kb spliced *myc*-containing RNA. We have further identified a 57K protein encoded by this RNA which we have implicated in transformation. The previously reported MH2 polyprotein P100, which has been shown to contain *gag* sequences, has been assumed to also contain *myc* information, in analogy to the MC29 P110 protein. By using a *pol*-specific probe, it has been shown that P100 does not contain any *pol* sequences (S. Collins et al., unpublished data) as was tentatively suggested by Neil et al. (15). It is likely that P100

does contain *myc*, in addition to a fragment of the *gag* gene. However, whatever the nature of MH2P100⁺, our data strongly suggest that this protein is not required for morphological transformation or oncogenicity. An oncogenic variant of MH2, MH2YS3, has been isolated which does not encode P100, but does encode a 2.6-kb mRNA which can be translated in vitro to yield p57^{myc}. Whereas uncloned MH2YS3 yields a complex pattern of *myc* RNAs, subclones of this virus (40 and 41, Fig. 4), have simpler RNA patterns and, in fact, synthesize predominantly 2.6-kb RNA. A majority of MH2YS3 viral subclones do not synthesize P100, but all encode the 2.6-kb mRNA. We, therefore, suggest that p57^{myc} is sufficient for transformation and that P100 may be an aberrant readthrough product, which does not necessarily contain the entire *v-myc* gene. We do not as yet have any data indicating what is the difference between the MH2P100⁺ and MH2YS3 genomes. Preliminary restriction enzyme analysis (data not shown) suggests that there is no detectable deletion in the MH2YS3 genome relative to MH2P100⁺, although there may be altered restriction enzyme sites within the genome.

It has been shown previously that there are biological differences between MC29 and MH2 (13). MH2 transforms cultured macrophages more efficiently, and fibroblasts less efficiently, than does MC29. More striking is the difference in oncogenicity after injection of immunologically competent 2- to 3-week-old quail. MC29 is only poorly oncogenic in these birds, whereas MH2 is highly oncogenic. Since the MC29-transforming protein contains *gag* sequences, whereas the MH2 protein does not, it is likely that MC29-transformed cells might be immunologically rejected via these *gag* determinants. Preliminary experiments support this contention. We have transformed quail macrophages in vitro from individual quail and injected the cells back into the autologous bird at 3 weeks of age. The MH2-transformed macrophages led to malignant tumors in two out of two birds whereas no tumors arose when identical numbers of MC29-transformed macrophages were injected into five different birds. This suggests that differences in oncogenicity do not involve a lack of available MC29 target cells and therefore implicates immune surveillance.

It has been shown by Hayward and his colleagues (11) that induction of bursal lymphomas involves transcriptional activation of the cellular *c-myc* gene. A 2.5- to 2.9-kb 5'-c-*myc* RNA has been detected in tumors as well as in the bursal tumor-derived cell line RP9. It is interesting to note that in RP9 cells the 2.9-kb *c-myc* RNA encodes a protein detected by in vitro translation with a size identical to MH2 p57^{myc} (C.

Pachl, W. Schubach, R. Eisenman, and M. Linial, manuscript in preparation). The relationship of the *v-myc* and *c-myc* encoded 57K proteins to MC29 P110^{gag-myc} and MH2 P100 is currently under investigation.

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