Haematology and Blood Transfusion Vol. 28
Modern Trends in Human Leukemia V
Edited by Neth, Gallo, Greaves, Moore, Winkler

The Transforming Gene of Avian Acute Leukemia Virus MC29

K. Bister, P. Enrietto, T. Graf, and M. Hayman

A. Introduction

The transforming gene of avian acute leukemia virus MC29 is represented by a hybrid structure of a partial complement from the structural gene *gag* and MC29-specific sequences, termed *myc*. As a genetic unit these sequences together code for the transforming protein p110 [1–3]. From the continuous MC29-transformed quail cell line Q10 [3] partially transformation-defective (*td*) mutants of MC29 have been isolated which have a strongly reduced ability to transform macrophages in vitro or to induce in vivo any of the tumors typically associated with wild-type (*wt*) MC29 [9]. These mutants are still able to transform fibroblasts in culture. It was recently shown that their altered oncogenic properties are due to the loss of transformation-specific sequences from the genomic RNAs and their protein products [5, 8]. On passage through chicken macrophages, one of these mutants, MC29 *td* 10H, gave rise to a virus, termed MC29 10H BI, which has regained the ability to transform macrophages efficiently [11].

In this communication we report on nucleotide-sequencing data which make possible a more precise location of the deletions in the *td* MC29 *myc* region, and we also show that MC29 10H BI RNA contains *myc* sequences that are not present in *td* MC29, but are shared with *wt* MC29 as well as with the cellular *c-myc* locus.

B. Results and Discussion

I. Genetic Structure of *td* Mutants of MC29

The basic genetic structure of the deletion mutants *td* 10A, *td* 10C, and *td* 10H is shown in Fig. 1. The genomic RNAs have lost overlapping *myc*-specific sequences of 200, 400, and 600 nucleotides, respectively. The deleted sequences are represented by *myc*-specific oligonucleotides 1, 7b, and 26, located 3′ to a *Sal*I site present in *wt* MC29 proviral DNA [5]. In order to locate these deletions more precisely we have sequenced *wt* MC29 proviral DNA and were able to locate *myc* oligonucleotides 26 and 1 near to the *Sal*I site and *myc* oligonucleotide 3 near to the *myc-env* junction (Fig. 2). All *td* mutant RNAs contain *myc*-oligonucleotide 3 and *env* oligonucleotides 7a, 14a, and 2 [5]. Hence, the *myc-env* junction is unaffected by the deletions and conserved in *td* and *wt* MC29 RNAs. All *td* mutant RNAs lack *myc* oligonucleotide 26 [5], and their proviruses lack a *Cla*I site present in *wt* MC29 proviral DNA ([6]; compare Fig. 2). The smallest deletion, in mutant *td* 10A, appears to end within the 5′ half of oligonucleotide 1, since 10A RNA was shown to contain a truncated version (1a) of this oligonucleotide, differing from the *wt* form by the lack of one U and one AAC residue upon digestion with RNase A [5]. This would place the 5′ origin of the deletion near the *Sal*I site. In excellent agreement with this the larger deletions in *td* 10C and *td* 10H result in the total loss of oligonucleotides 1 and 7b, which apparently maps 3′ from 1 and was found in *td* 10A RNA [5].

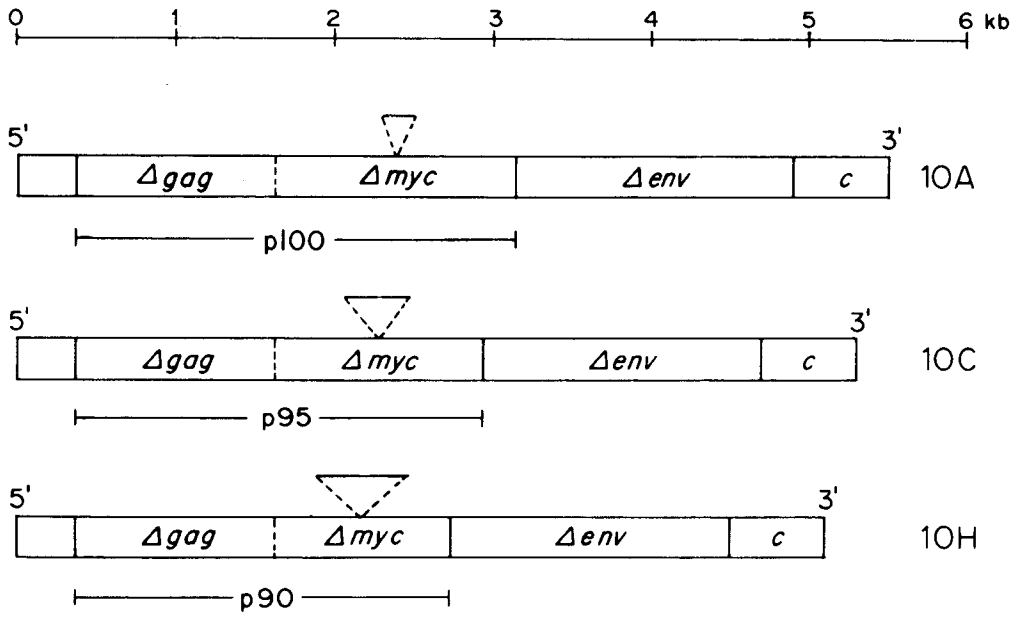

Fig. 1. Schematic diagram of the genomic RNAs and gene products of MC29 deletion mutants 10A, 10C, and 10H. *Δ* indicates the presence of partial complements of the structural genes *gag* and *env*, and of the transformation-specific sequences *myc*. Complexities of RNAs and genes are given in kilobases (kb), those of the *gag-myc* hybrid proteins (p) in kilodaltons

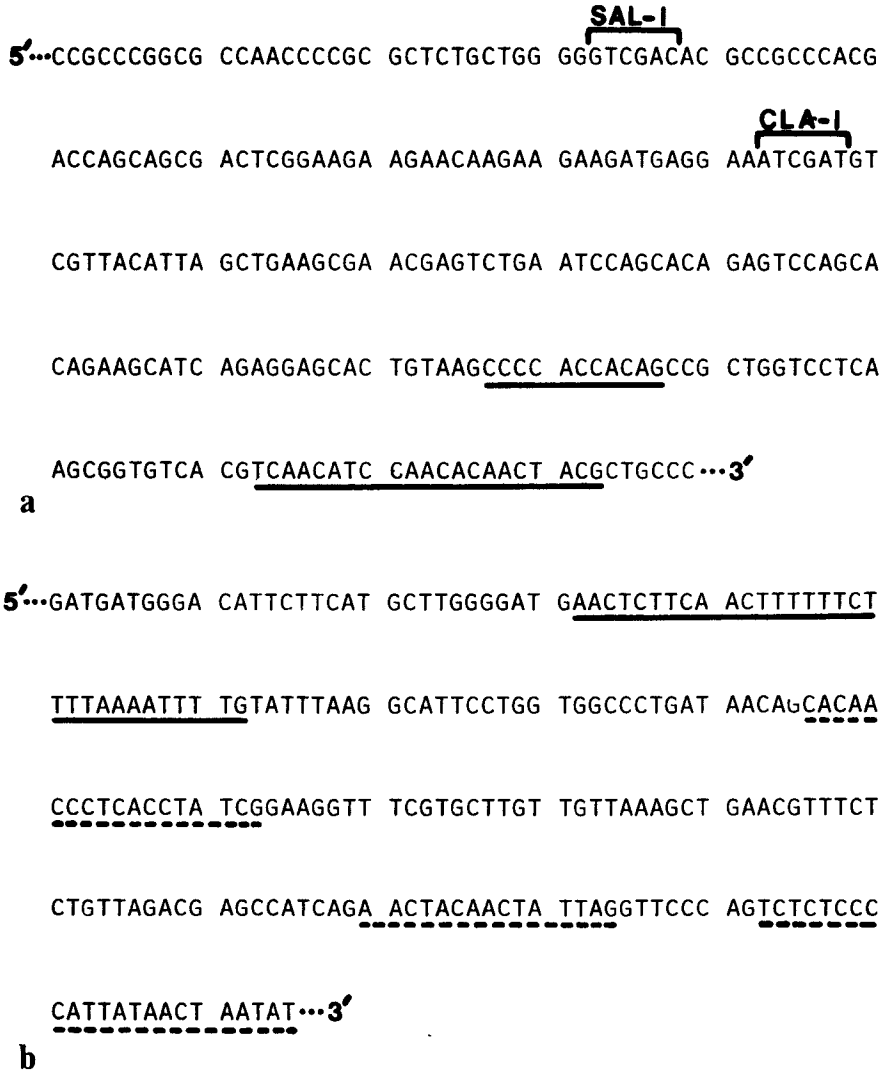

Fig. 2a, b. Nucleotide sequence analysis of *wt* MC29 proviral DNA around the *SalI* and *ClaI* sites in the center of the *myc* region **a** and around the *myc-env* junction **b**. **a** A subcloned 1.5-kb *PstI* fragment of cloned MC29 DNA [13] was sequenced from both the *SalI* and the *ClaI* sites by the method of Maxam and Gilbert [7]. Sequences corresponding to previously identified *myc*-specific T_1-oligonucleotides 26 and 1 (in the 5′ to 3′ order) are underlined [1, 5]. **b** A subcloned 1.1-kb *SalI-Bam*HI fragment of cloned MC29 DNA [5] was sequenced from the *Bam*HI site. Sequences corresponding to previously identified *myc*-specific T_1-oligonucleotide 3 are indicated by a *solid line*; those corresponding to *env* T_1-oligonucleotides 7a, 14a, and 2 (in the 5′ to 3′ order) are indicated by *broken lines*

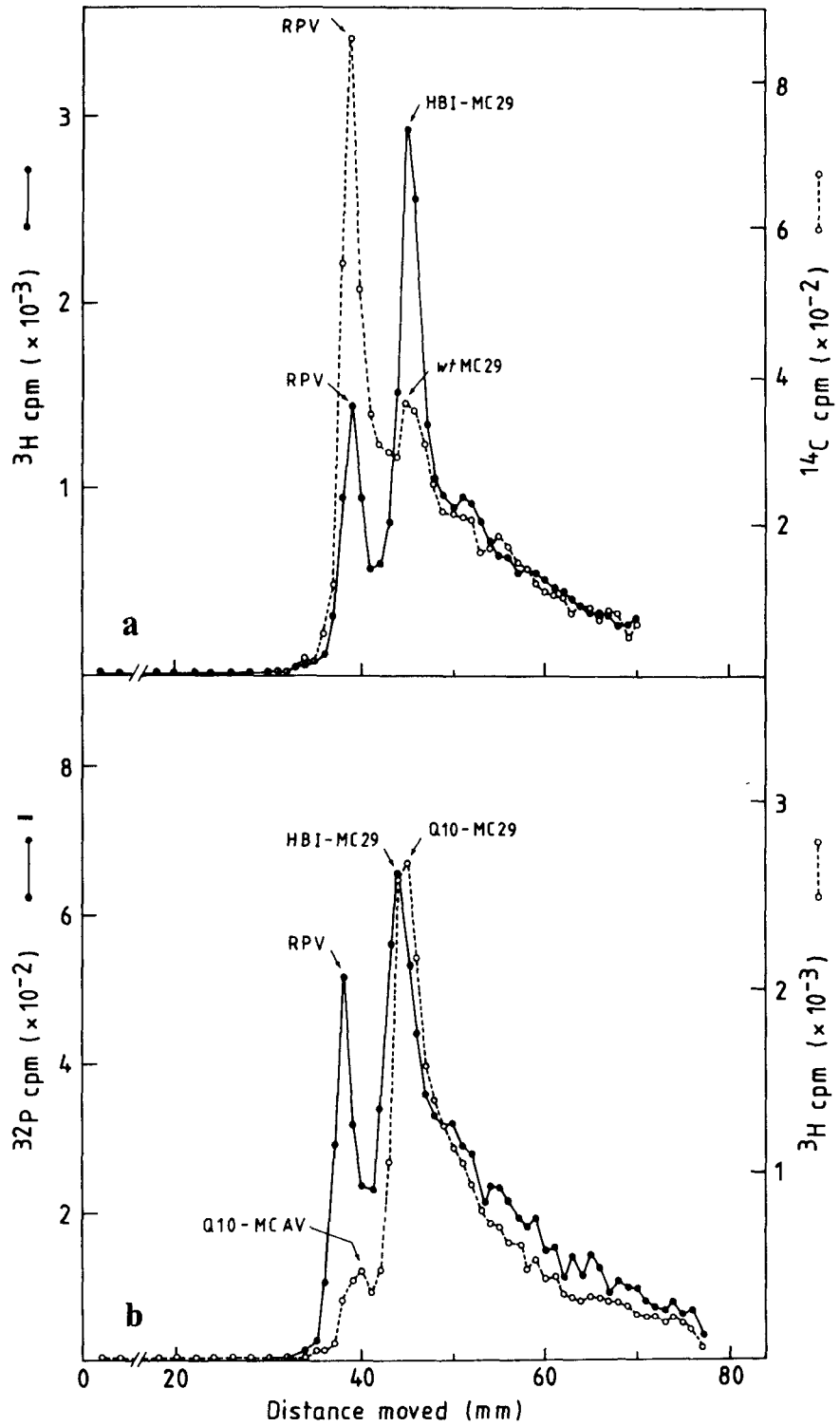

Fig. 3a, b. Electrophoresis in 2.1% polyacrylamide gels of the RNA monomers of **a** [^{3}H] or **b** [^{32}P] 10H BI (RPV) in the presence of **a** [^{14}C]RNA of *wt* MC29 (RPV), rescued from the nonproducer line Q8, or **b** [^{3}H]RNA of Q10-MC29 (Q10-MCAV). Electrophoresis was at 50 V for 4 h and otherwise as described [5]

These data allow the following conclusions: (1) The deletions in all three *td* mutants are overlapping and probably even start from the same map position near the *SalI* site. (2) Since the *SalI* site maps about 2050 nucleotides (corresponding to protein sequences of 78,000 daltons) 3′ from the initiation site for the synthesis of the *gag*-related transforming proteins [5], it appears almost certain that the deletions are in phase and that the transforming proteins (p100, p95, p90) of the *td* mutants share the carboxy-terminal sequences with the *wt* protein (p110). This is also strongly supported by the fact that the sizes of the deleted RNA sequences correspond closely to the smaller sizes of these proteins (Fig. 1). (3) Since all mutants were selected for fibroblast transformation, it appears that the 5′ or 3′ terminal *myc* sequences, or both, are needed for that activity, but not the central *myc* sequences. Their deletion,

however, strongly affects the potential for hematopoietic cell transformation. This could possibly reflect the presence of two functional domains on the *wt* p110, one of which would be deleted in the mutant proteins. A possibly important difference between the *wt* and the mutant proteins is that p110 is strongly phosphorylated whereas the mutant proteins have lost specific threonine phosphorylation sites [4, 10].

II. Genetic Structure of Recovered MC29 10H BI

When *td* MC29 10H was passaged through chick macrophage cultures, a virus (10H BI) was recovered that had regained the ability to transform macrophages as efficiently as *wt* MC29 [11]. Here, we used a nonproducer line of 10H BI-transformed quail embryo fibroblasts that was superinfected with ring-necked pheasant virus (RPV) and labeled with [^{3}H]uridine or $H_3{}^{32}PO_4$. Virus was purified and electrophoretic analysis of purified, heat-denatured viral RNA showed the presence of two components: the 8.5-kb RNA of RPV and a 5.7-kb RNA, comigrating with *wt* MC29 RNA originating from MC29-transformed cell lines Q8 or Q10 (Fig. 3). Hence, 10H BI RNA is 0.6 kb larger than the genomic RNA of the deletion mutant *td* MC29 10H (Fig. 1) from which it was derived on passage through macrophages. To analyze the structure and possible origin of these acquired sequences, T_1-oligonucleotide fingerprints of [^{32}P] RNA were prepared. [^{32}P] 10H BI (RPV) RNA was hybridized with cloned proviral MC29 DNA from the *Δgag-myc* or the *myc-Δenv* region [5], or with cloned *c-myc* DNA [12]. Figure 4 shows that the oligonucleotide pattern of 10H BI RNA from the *myc* and the adjacent *gag* and *env* regions is very similar to that of *wt* MC29 RNA reported previously [5]. In particular, *myc* oligonu-

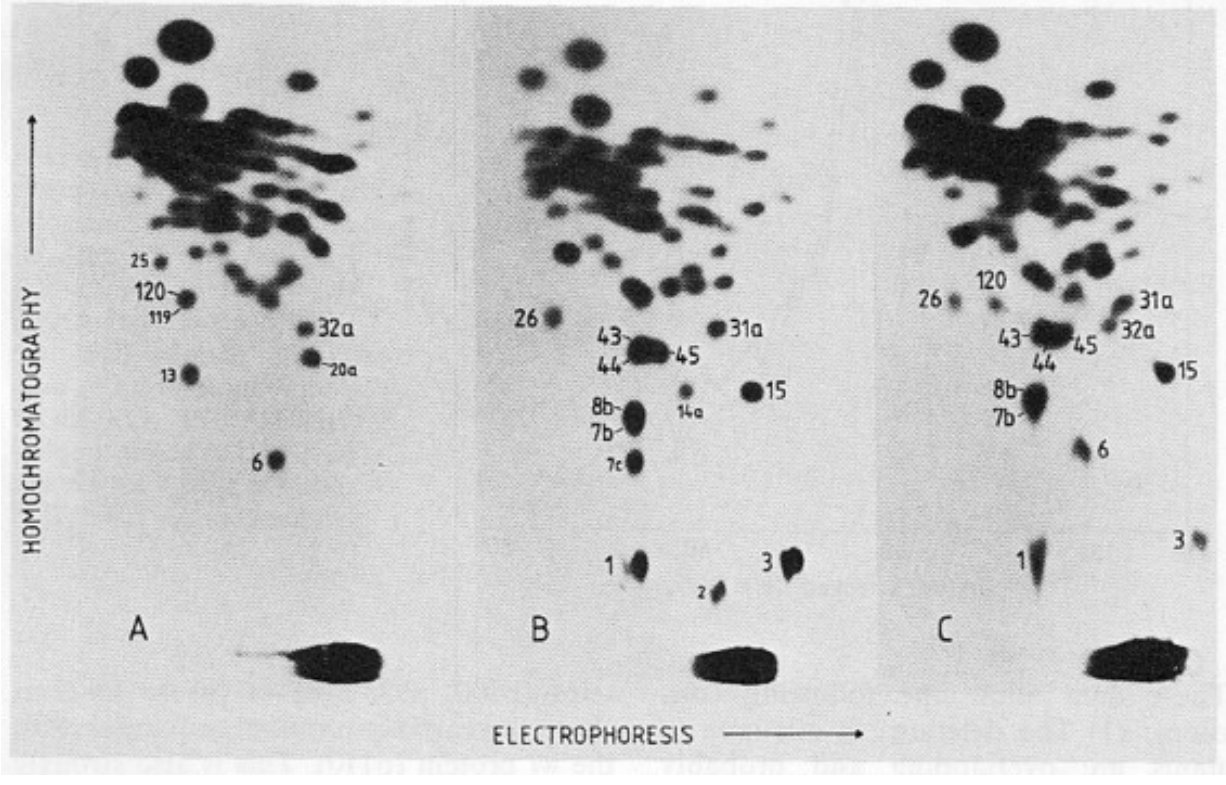

Fig. 4A–C. Fingerprint analysis of [^{32}P]RNA from 10H BI (RPV) hybridized with (**A**) DNA from pBR322 containing the 5′ half of the *myc* sequence and adjacent *gag* sequences (*pmyc*-5′; see [5]), (**B**) DNA from pBR322 containing the 3′ half of the *myc* sequence and adjacent *env* sequences (*pmyc*-3′; see [5]), or (**C**) DNA from phage Charon 4A containing the cellular *c-myc* locus [12]. Hybridization of [^{32}P]RNA with plasmid or phage DNA, isolation of the hybrid, and fingerprint analysis of hybridized RNA were carried out as already described [5]. The composition of T_1-oligonucleotides after digestion with RNase A was determined. Oligonucleotides with previously found compositions were numbered accordingly [5]. The composition of 7c is: 2U, 6C, 2AC, AU, AG, AAC. *Large numbers* indicate *myc* oligonucleotides; *small numbers* refer to *gag* or *env* oligonucleotides

cleotides 1, 7b, and 26, which are missing from *td* MC29 10H RNA are present in 10H BI RNA. Oligonucleotide 7c appears to be a variant of *env* oligonucleotide 7a present in *wt* and *td* 10H MC29 [5]. There are more differences in the oligonucleotide pattern of *td* 10H and 10H BI RNAs (not shown). These would have to be explained by point mutations or recombination with helper virus, if 10H BI is directly derived from *td* 10H in a recombinational event involving cellular *c-myc* sequences. Due to the close relationship between the viral and the cellular *myc* sequences [12, 13], such an event would involve double legitimate recombination, and hence could conceivably have occurred during the generation of 10H BI.

Acknowledgment

We wish to thank J. Arrand and P. Bullock for invaluable advice on the DNA sequencing, and Lorrine Chao for technical assistance.

References

1. Bister K, Duesberg PH (1980) Genetic structure of avian acute leukemia viruses. Cold Spring Harbor Symp Quant Biol 44:801–822
2. Bister K, Duesberg PH (1982) Genetic structure and transforming genes of avian retroviruses. In: Klein G (ed) Advances in viral oncology, vol 1. Raven, New York, pp 3–42
3. Bister K, Hayman MJ, Vogt PK (1977) Defectiveness of avian myelocytomatosis virus MC29: Isolation of long-term nonproducer cultures and analysis of virus-specific polypeptide synthesis. Virology 82:431–448
4. Bister K, Lee W-H, Duesberg PH (1980) Phosphorylation of the nonstructural proteins encoded by three avian acute leukemia viruses and by avian Fujinami sarcoma virus. J Virol 36:617–621
5. Bister K, Ramsay GM, Hayman MJ (1982) Deletions within the transformation-specific RNA sequences of acute leukemia virus MC29 give rise to partially transformation-defective mutants. J Virol 41:754–766
6. Enrietto PJ, Hayman MJ (1982) Restriction enzyme analysis of partially transformation defective mutants of the acute leukemia virus MC29. J Virol 44:711–715
7. Maxam AM, Gilbert W (1980) Sequencing end-labeled DNA with base-specific chemical cleavages. In: Grossmann L, Moldave K (eds) Methods in enzymology, vol 65. Academic Press, New York, pp 499–560
8. Ramsay GM, Hayman MJ (1982) Isolation and biochemical characterization of partially transformation-defective mutants of avian myelocytomatosis virus strain MC29: Localization of the mutation to the *myc* domain of the 110,000-dalton *gag-myc* polyprotein. J Virol 41:745–753
9. Ramsay GM, Graf T, Hayman MJ (1980) Mutants of avian myelocytomatosis virus with smaller *gag* gene-related proteins have an altered transforming ability. Nature 288:170–172
10. Ramsay GM, Hayman MJ, Bister K (1982) Phosphorylation of specific sites in the *gag-myc* polyproteins encoded by MC29 type viruses correlates with their transforming ability. EMBO J 1:1111–1116
11. Ramsay GM, Enrietto PJ, Graf T, Hayman MJ (to be published) Recovery of *myc* specific sequences by a partially transformation defective mutant of avian myelocytomatosis virus, MC29, correlates with the restoration of transforming activity. Proc Natl Acad Sci USA
12. Robins T, Bister K, Garon C, Papas T, Duesberg PH (1982) Structural relationship between a normal chicken DNA locus and the transforming gene of the avian acute leukemia virus MC29. J Virol 41:635–642
13. Vennström B, Sheiness D, Zabielski J, Bishop JM (1982) Isolation and characterization of *c-myc*, a cellular homologue of the oncogene (*v-myc*) of avian myelocytomatosis virus strain 29. J Virol 42:773–779