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NOTES

Two Virus-Specific RNA Species Are Present in Cells Transformed by Defective Leukemia Virus OK10

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OK10 is a defective leukemia virus which shares some biological and biochemical properties of avian myelocytomatosis virus (MC29). We investigated the pattern of transcription of OK10 in both quail and chicken cells. In both cell types, OK10 produced two polyadenylated RNA species of 8.6 and 3.5 kilobases, which both contained sequences derived from the 5′ end of the genome and also the presumed transforming gene (*myc*). This is a novel form of expression for defective leukemia viruses of the MC29 subgroup and may indicate that there is an as-yet-unidentified protein produced in OK10-infected cells which may be involved in transformation.

OK10 is a defective leukemia virus (DLV) which causes a variety of neoplasms, including carcinomas, in vivo (15) and is capable of transforming macrophages and fibroblasts in vitro (11). As with other DLVs, the oncogenicity of OK10 is attributed to nucleotide sequences derived from a normal cellular gene which have been acquired by the virus at the expense of parts of the viral structural genes. In view of its biological properties, OK10 was classified as a member of the avian myelocytomatosis virus (MC29) subgroup of DLVs (11). This was subsequently confirmed by nucleic acid hybridization analysis (21). These analyses have shown that the cellular sequences acquired by OK10 share extensive homology with those of MC29, termed *myc* (previously called *mac* or *mcv*). However, unlike MC29, cells infected with OK10 in the absence of helper virus produce virus-like particles (18). These particles are noninfectious due to lack of both reverse transcriptase activity and envelope glycoproteins (18) and contain an 8.6-kilobase (kb) RNA species (6). In addition, two protein products have been detected using antisera against viral structural proteins—pr76, the *gag* gene precursor, and p200, a polyprotein containing all of the *gag* sequences, most of *pol*, and some additional information which was presumed to be derived from *myc* (18). These data, together with a recent study of the T_1 oligonucleotides of the genomic RNA (6), have indicated that the genome of OK10 has an unusual structural organization compared to other DLVs. The *myc*-specific sequences have been inserted between the *pol* and *env* genes, both of which have been partially deleted. Thus, the gene order has been deduced to be: 5′ *gag* Δ*pol* *myc* Δ*env* 3′.

In view of this unique genome organization, we investigated the pattern of transcription of the OK10 sequences in cells infected by the virus. The polyadenylic acid-containing RNA was extracted from OK10 QB5 cells, a line of OK10-transformed quail cells which produce noninfectious virions, and analyzed by electrophoresis and blot hybridization. Using a probe representative of the complete MC29 viral genome (Fig. 1a), two RNA species were detected. The larger 8.6-kb RNA probably represents the OK10 genome; however, the smaller 3.5-kb RNA was not previously reported and is possibly a subgenomic mRNA. To investigate this further, we examined these RNAs using probes specific for different parts of the viral genome. Both species hybridize to probes representing either the *myc*-specific or 5′ regions of the viral genome (Fig. 1b and c). Since the 3.5-kb RNA contains polyadenylic acid, we consider it to be a subgenomic mRNA encoding *myc* sequences spliced to a 5′ leader. Since the QB5 cell line was derived from a cloned line of OK10-infected quail fibroblasts, we considered the possibility that the pattern of transcription in this cell line was peculiar. The defective OK10 was therefore rescued from these with the replication-competent virus OK10-associated virus (OK10 AV), and the virus complex was used to infect secondary chicken embryo fibroblasts (CEF). RNA was extracted from these cells, and also from control

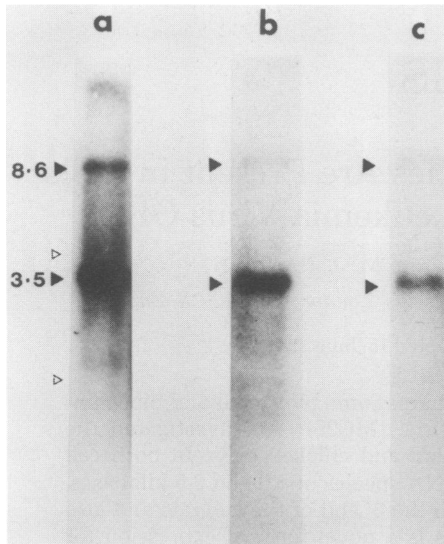

FIG. 1. *Virus-specific RNA species in OK10-transformed quail cells. The origin and culture of cells were as previously described (18, 22). RNA was extracted from whole cells by lysis in NTE (0.1 M NaCl, 0.01 M Tris [pH 7.4], 1 mM EDTA) containing 1% sodium dodecyl sulfate (SDS) and 0.5% sodium deoxycholate. The lysate was extracted twice with phenol and twice with chloroform-isoamyl alcohol (24:1). After ethanol precipitation, polyadenylic acid-containing RNA was selected on oligodeoxythymidylate cellulose (Miles Ltd.) (3). The RNA was analyzed by electrophoresis on 1.2% agarose gels containing 10 mM methylmercuric hydroxide (4) and transferred to diazophenylthioether paper (B. Seed, personal communication) in 20 mM citrate phosphate buffer (pH 4.0) (1). Virus-specific RNAs were detected by hybridization at 41°C in 50% formamide, 5× SSC (1× SSC = 0.15 M sodium chloride–0.015 M sodium citrate), 50 mM N-2-hydroxyethyl-piperazine-N′-2-ethanesulfonic acid (pH 7.0), 0.1% SDS, 200 μg of denatured and sonicated calf thymus DNA per ml (Sigma Ltd.), 10% dextran sulfate (24), and 1× Denhardts (9) at 5 ng per ml of probe. Filters were washed in 2× SSC–0.1% SDS, followed by 0.1× SSC–0.1% SDS at 45°C and exposed for autoradiography with intensifying screens at −70°C. For repeated analysis of the same filter, hybridized probes were removed by washing in 99% formamide at 65 to 80°C and monitored for complete elution by autoradiography. All the probes used in this study were prepared by nick translation (9) of molecularly cloned DNA sequences kindly provided by B. Vennstrom and J. M. Bishop (San Francisco, Calif.). Growth of recombinant plasmids was performed in accordance with the guidelines of the U.K. Genetic Manipulation Advisory Group. The probes were: (i) pMC 38 (23) containing the whole DNA provirus of MC29; (ii) MYC, a 1.5-kb Pst-1 fragment of pMC38 containing 80% of the myc sequences (20, 23) which maps approximately between 2.3 kb and 3.8 kb from the 5′ end of the MC29 genome; and (iii) 5′, a fragment from the Hin F1 site at nucleotide 970 in Czernilofsky et al. (7) to the Sac-1 site of pSRA-2 (8). This fragment is approximately 200 base pairs long and starts 55 nucleotides from the 5′ end of the genome. The figure shows 2.6 μg of RNA from OK10 QB5 cells (except track c where 20 μg of RNA was loaded) hybridized to pMC38 (a), myc (b), and 5′ (c) probes. The molecular weight (in kilobases) were derived using rRNA markers whose position is shown (▶).*

CEF infected with OK10 AV only, and analyzed as described above. Figure 2b shows the results of hybridization with a 5′-specific probe to RNA derived from OK10 AV-infected cells. OK10 AV has the typical genome structure of a replication-competent avian leukosis virus (6), and therefore we presume that the 8.5-kb RNA represents the virus genome and the 3.2-kb RNA represents the subgenomic *env* mRNA. The same two RNA species are detected in the cells infected with rescued OK10 (Fig. 2a), because the ratio of helper virus to OK10 is high (10 to 100:1) and any hybridization to OK10 RNA is masked by the helper virus RNA present. However, if *myc*-specific probe is used on RNA derived from the same cells, a number of different RNA species are detected (Fig. 2c and d). The 3.2-kb and 8.5-kb helper virus RNA species are not detected (Fig. 2d), confirming the specificity of the probe. However, in the cells infected with rescued OK10 (Fig. 2c), RNA species are detected which comigrate (unpublished data) with the 8.6-kb and 3.5-kb RNAs detected in OK10 QB5 cells. These results show that these two RNA species are a property of the OK10 genome since they are expressed in both quail and chicken cells. The 2.2-kb RNA species detected in both CEF cultures with the *myc*-specific probe (Fig. 2c and d) is derived from endogenous *myc*-specific sequences expressed in uninfected CEF (unpublished data).

The results described above suggest that the strategy employed by OK10 for the expression of oncogenicity may be significantly different from the related virus MC29. Cells infected with MC29 contain only a single genomic-sized RNA species (20) which acts as mRNA for the synthesis of a 110,000-dalton polyprotein (p110) containing both *gag* and *myc* determinants (5, 14). The isolation of transformation-defective mutants of MC29 which encode smaller polyprotein (17) has implicated the p110 in transformation. With OK10, on the other hand, the *gag* gene appears to be complete in that a normal pr76^{gag} precursor can be detected in infected cells. The *myc* sequences are instead expressed, at least in part, in a 200,000-dalton polyprotein (p200) which contains *gag*, *pol*, and *myc* determinants. The synthesis of both the pr76^{gag} and

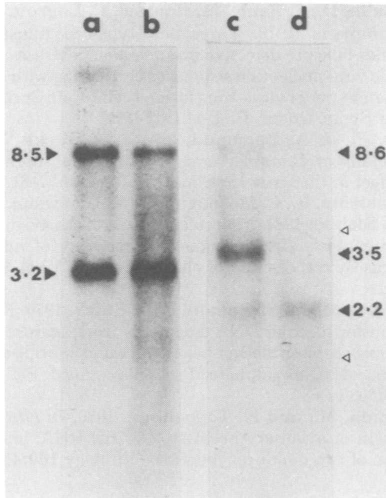

FIG. 2. *Virus-specific RNA species in OK10 and OK10 AV-infected CEF. RNA extracted, electrophoresed, and transferred as in Fig. 1. RNA from OK10 AV-infected CEF in tracks b (2.0 µg) and d (17 µg) and from CEF infected with a mixture of OK10 and OK10 AV in tracks a (1.4 µg) and c (11 µg). These were probed with 5'- (a and b) or myc- (c and d) specific probes and visualized by autoradiography.*

p200 can be directed by the 8.6-kb genome-sized RNA in vitro (unpublished data). However, no potential protein product from the 3.5-kb subgenomic RNA has yet been identified.

Our data indicate that the 3.5-kb RNA contains a leader sequence derived from the 5' end of the genome as well as some, or possibly all, of the *myc* sequences. One possibility for the generation of the RNA is that the 5' leader has been spliced to a coding region at a point beyond the termination codon of p200, giving rise to an independently initiated protein encoded in the 3' region of *myc*. Alternatively, the splice joint could lie within the coding region of p200 in the same or another reading frame. Consideration of the structure of the OK10 genomic RNA (5, 17; and see above) implies that this smaller RNA may also contain a residual part of the *env* gene and all of the so-called *c* region. Thus, any protein product from the subgenomic RNA could include *env*- as well as *myc*-related determinants. However, no protein precipitable by antiglycoprotein serum has yet been detected in virus-transformed cells (18).

OK10, like MC29 and other DLVs, has a pluripotent oncogenic spectrum in vivo and in vitro (10, 15). It is therefore conceivable that the two intracellular RNAs may encode proteins responsible for different aspects of this oncogenicity. In this regard, the expression of the *myc* sequences from the OK10 genome may be analogous to the situation observed with cells transformed by avian erythroblastosis virus in which two intracellular RNAs are also detected (2, 20). These RNAs are thought to encode two distinct protein products, p75 (12) and p41 (13, 16, 25), both of which contain determinants derived from avian erythroblastosis virus oncogene.

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