Differences between the Ribonucleic Acids of Transforming and Nontransforming Avian Tumor Viruses*

Peter H. Duesberg and Peter K. Vogt

DEPARTMENT OF MOLECULAR BIOLOGY AND VIRUS LABORATORY, UNIVERSITY OF CALIFORNIA, BERKELEY, CALIF. 94720; AND DEPARTMENT OF MICROBIOLOGY, UNIVERSITY OF WASHINGTON SCHOOL OF MEDICINE, SEATTLE, WASH. 98105

Abstract. The 60-70S RNAs of several transforming and nontransforming avian tumor viruses have different electrophoretic mobilities. The RNA of transforming viruses contains two electrophoretically separable subunit classes: *a* and *b*. The relative concentrations of these subunits vary with the virus strain. Avian leukosis viruses and nontransforming derivatives of a sarcoma virus lack subunits of class *a*. It is suggested that the presence of the class *a* subunit is related to the transforming ability for fibroblasts of the virus.

Avian RNA tumor viruses show great variety as pathogens and antigens.[1] Some are highly virulent, killing the majority of the infected animals; others can establish latent infections which persist throughout the lifetime of the host without causing disease. The tumors induced by avian RNA tumor viruses may be broadly classified as sarcomas and leukemias. Sarcoma-causing viruses also transform chick embryo fibroblasts in tissue culture, whereas, with some notable exceptions, leukemogenic viruses are far less efficient in neoplastic transformation of fibroblasts *in vitro*.[2,19] Despite this diversity of biological properties most of the proteins of avian RNA tumor viruses cannot so far be distinguished with physical techniques.[3] Only the glycoproteins from various avian RNA tumor virus types have been differentiated from each other by gel electrophoresis.[4] There has been no direct comparison of the physical properties of 60-70S RNAs of different virial strains.

The nucleic acids of RNA tumor viruses consist of two major RNA components, fast-sedimenting 60-70S RNA and a slowly-sedimenting 4S RNA.[5] The fast-sedimenting RNA has an aggregate structure and can be dissociated by heat or dimethylsulfoxide into several major subunits with molecular weight of about 3×10^6 and variable amounts of smaller, heterogeneous RNAs.[3,6-10] Since both the absolute RNA content per average virion and the molecular weight of the 60-70S RNA had been estimated to be $10-12 \times 10^6$[5,11] it was proposed that the virion contains one RNA complement of 60-70S composed of 3-4 subunits of 3×10^6 daltons each.[3] It has not yet been determined whether the subunits differ in their physical or chemical structure.

Recently it was found that after exposing avian sarcoma viruses to γ- or UV-irradiation, surviving viruses may be found which can still reproduce but fail to transform chicken fibroblasts.[12,15] The nontransforming agents are anti-

genically identical to the transforming sarcoma virus from which they are derived, presumably by a radiation-induced genetic lesion. These observations suggest that the nontransforming variants might differ from their transforming progenitors in the structure of their RNAs—perhaps by deletion of a portion of the RNA—and that such a difference, if detectable, might be related to the transforming ability of the virus. The present report provides evidence that the RNAs of a transforming avian sarcoma virus and its nontransforming derivative differ, both in their native form and after heat dissociation into subunits. Similar differences were also detected when the RNA complements of other avian sarcoma and leukosis viruses were compared.

Materials and Methods. Viruses: Avian sarcoma virus B77 was isolated from a spontaneous fibrosarcoma of a chicken.[13] B77 belongs to avian tumor virus subgroup C.[14] B77 was cloned serially before use and appeared to be free of associated, nontransforming viruses. After irradiation of B77 with UV light, several isolates of a nontransforming agent with the envelope properties of B77 were made. These nontransforming agents will be referred to as NT B77 clones no. 1–5.[15] Other avian sarcoma viruses used in this study were the Schmidt-Ruppin strain of Rous sarcoma virus of subgroup A[16] (SR RSV-A), the Prague strain of Rous sarcoma virus of subgroup C (PR RSV-C), and Rous sarcoma virus type O [RSV(O)]. Cloned stocks of SR RSV-A or PR RSV-C contain at most small quantities of nontransforming viruses. RSV(O) may contain an associated, nontransforming virus.[26] The representative avian leukosis viruses were avian myeloblastosis virus (AMV) which contains members of subgroups A and B,[16] myeloblastosis-associated virus of subgroup B (MAV-B), and Carr Zilber-associated virus of subgroup D[14] (CZAV). All viruses with the exception of AMV were produced in type C/O, C/A, or C/B[17] chick embryo fibroblast cultures, according to published techniques, in 10-cm plastic Petri dishes containing 10 ml of medium.[18] The growth medium consisted of medium 199 (GIBCO Inc.) supplemented with 5% calf serum, 1% chicken serum, 10% tryptose phosphate broth and 0.5 μg/ml fungizone. AMV was grown in suspension cultures of myeloblasts obtained from leukemic chickens, using the medium described by Baluda.[19] All media contained 1% dimethylsulfoxide which increased virus yield and improved the appearance of the cells in prolonged culture.[20]

Labeling of Viral RNA: To grow [³H]RNA-virus, 50–150 μCi[³H]uridine (20 Ci/mmol, New England Nuclear) was added directly to the medium of an infected culture for 10–12 hr before virus harvest. Labeling with ³²P was also for 10–12 hr; 1 mCi of carrier-free [³²P]H₃PO₄ was added to a virus-producing culture in 6 ml of medium consisting of phosphate-free medium 199 (GIBCO Inc., Berkeley, Calif.) supplemented with 1.5% tryptose broth, 1% calf serum, 1% chicken serum, 1% dimethylsulfoxide, 0.5 μg/ml fungizone and 0.2% NaHCO₃.

Purification of labeled virus; isolation and analysis of viral RNA: Avian tumor viruses were purified and viral RNA was isolated by published procedures.[6,21] Additional pronase treatment had no effect on the properties of the RNA described here. Gel electrophoresis was in 2% diacrylate-crosslinked polyacrylamide gels as described previously[6,22] with the following modification. Gel monomer solution contained (per 100 ml) 30 g of deionized acrylamide and 2.7 g of diacrylate and was stored at $-20°$C to prevent hydrolysis of diacrylate. 1-mm gel sections (gel slicer: Diversified Scientific Instruments, San Leandro, Calif.) were dissolved during 20 min at 20°C in 50 μl of 2 M piperidine and radioactivity was determined after the addition of 2 ml of 20% NCS (Nuclear Chicago) in toluene-based scintillation fluid.

Results. Coelectrophoresis of the RNAs from four avian sarcoma viruses, before and after heat-dissociation: The 60–70S RNAs of SR RSV-A, B77, PR RSV-C and RSV(O) were isolated by the phenol–sodium dodecyl sulfate method and prepared by sucrose gradient sedimentation[6] (inserts of Fig. 1A, C, and E).
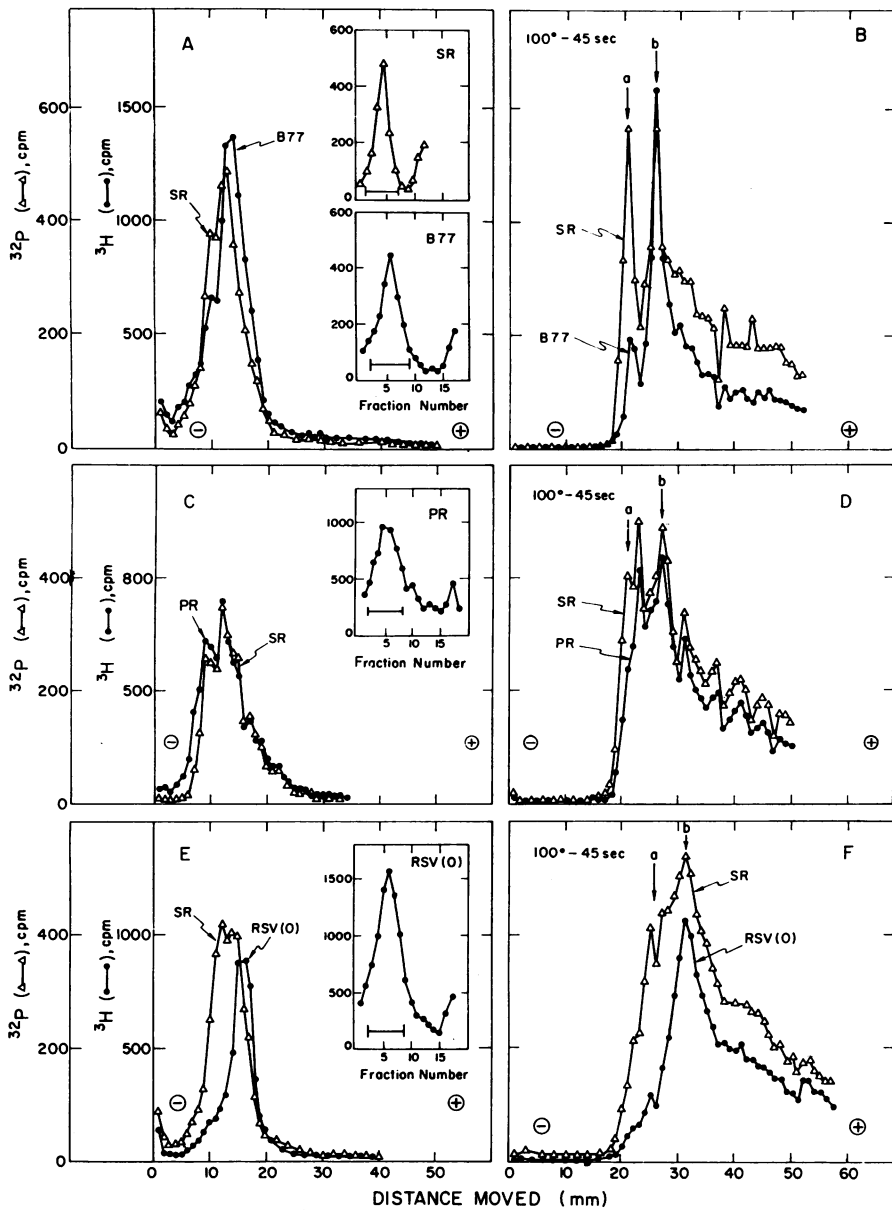
FIG. 1.   Electropherograms of the 60–70S RNAs of SR RSV-A compared with those of B77, PR RSV-C and RSV(O) before (*A, C, E*) and after (*B, D, E*) heat-dissociation. Heat dissociation was in 20–30 μl 1 mM Tris·HCl (pH 7.4), 1 mM EDTA, 0.2% sodium dodecyl sulfate and 10% glycerol in a sealed ampule for 45 sec at 100°C followed by rapid cooling in melting ice.   Electrophoresis was for 6 hr at 7 V/cm as described in *Materials and Methods*. Final sucrose gradient preparations of the 60–70S radioactive RNAs are shown in the insert of Fig. 1*A, C*, and *D*.   Sedimentation was in a linear sucrose gradient 10–25% (w/v) sucrose containing 0.1 M NaCl–0.01 M Tris·HCl (pH 7.4)–1.5 mM EDTA–0.1% sodium dodecyl sulfate for 45 min at 65,000 rpm in a Spinco SW65 rotor at 20°C.   The fractions indicated by the bar were pooled and precipitated with ethanol, with 10-μg tobacco mosaic virus RNA as carrier for electrophoretic analysis.

The results of coelectrophoresis of several combinations of these RNAs are shown in Fig. 1*A*, *C*, and *E*. RNAs obtained from SR RSV-A and PR RSV-C have the lowest electrophoretic mobility. The RNAs of B77 and RSV(O) have relatively higher mobilities. Since the 60–70S RNAs consist of several RNA subunit components, the observed electrophoretic differences may have at least two explanations: (1) The subunits themselves could show viral-type-specific differences in electrophoretic mobility. Thus, the subunits of RSV(O) could migrate faster than those of SR RSV-A. (2) Subunits could be of several electrophoretically separable classes with each class present in all viral types. Viral-type-specific differences could then result from variations in the relative concentrations of various subunits. To help us decide between these possibilities, the 60–70S RNAs were dissociated by heat and coelectrophoresed in polyacrylamide gels as shown in Fig. 1*B*, *D*, and *F*. In earlier studies, the heat-dissociated RNA of the Bryan strain of RSV[6] was resolved into one major component, of about 3×10^6 daltons and various amounts of heterogeneous, faster migrating RNAs whose concentration was found to increase with prolonged labeling periods of the virus.[3,7,9,10] The heat-dissociated RNAs of SR RSV-A, PR RSV-C, B77, and RSV(O), however, were resolved into two major components which were called class *a* and *b* subunits in the order of their increasing electrophoretic mobility. In some experiments, class *a* subunits of SR RSV-A, for example, appeared to be resolved further into two components (see Figs. 1 and 2). Separation of these components, however, was not reproducible.

It appears that SR RSV-A, PR RSV-C, B77, and RSV(O) all contain class *a* and *b* RNA in different relative concentrations. SR RSV-A shows the highest concentrations of class *a* RNA relative to class *b* RNA, and PR RSV-C, B77, and RSV(O), in this order, contain less *a* than *b*. Thus, in contrast to the electropherograms of the native RNAs, no differences in relative electrophoretic mobilities were detectable in the electropherograms of melted RNAs. These results indicate that the RNAs of SR RSV-A, PR RSV-C, B77, and RSV(O) differ primarily in their relative content of the common subunit classes, *a* and *b*. Although the respective RNA subunits of different avian tumor viruses have almost indistinguishable electrophoretic mobilities, it should be emphasized that they are assumed not to have identical base sequences.

Since stocks of avian sarcoma viruses grown in different genetic types of host cells always contained the two classes of subunits in their RNA complement, the occurrence of these subunits does not appear to be influenced by the genetic type of the host. However, some variations in the relative concentration of class *a* and *b* RNA were observed between different working stocks of the same virus, such as SR RSV-A (see Figs. 1 and 2). It is therefore not possible to say to what extent the differences in relative concentrations of RNA subunit classes observed with the four avian sarcoma viruses are under viral genetic control.

The RNA complements of AMV, CZAV, and MAV-B compared to that of SR RSV-A: Fig. 2 represents an electrophoretic comparison of native and heat-dissociated RNAs obtained from a transforming virus, SR RSV-A, and two non-transforming viruses, AMV and CZAV. The native RNAs of SR RSV-A and AMV, as well as CZAV, can be easily distinguished by polyacrylamide gel electro-
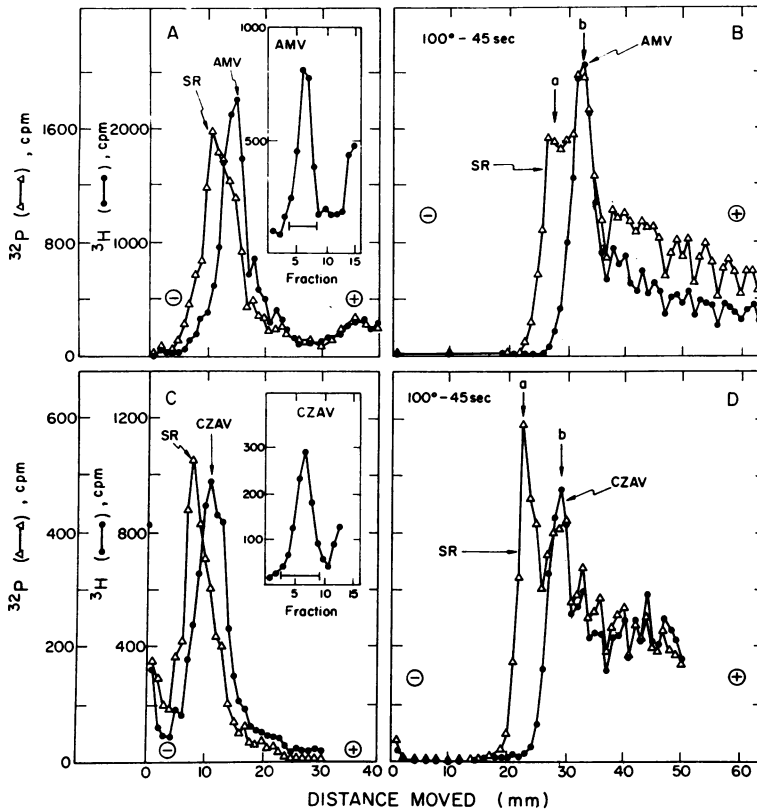
FIG. 2. Coelectrophoresis of the 60–70S RNAs of SR RSV-A with those of AMV and CZAV before (*A,C*) and after (*B,D*) heat-dissociation. Conditions as described for Fig. 1. Preparation of the 60–70S RNAs is illustrated in the inserts of Fig. 2*A* and *C*.

phoresis, in the same way as the native RNAs of avian sarcoma viruses recorded in Fig. 1. However, in contrast to the patterns obtained with SR RSV-A, the dissociated RNAs of AMV and CZAV lack the class *a* subunits. A lack of the class *a* subunits was also observed with the nontransforming MAV-B (data not shown). This difference may reflect the main biological distinction between SR RSV-A, a sarcoma virus with high transforming efficiency for fibroblasts, and AMV, CZAV, and MAV-B—viruses that lack transforming ability for fibroblasts under conditions that allow transformation by RSV. On the other hand, SR RSV-A and the leukosis viruses included in this experiment belong to different subgroups and are not antigenically identical.[16] Thus, the differences in RNA electrophoretic patterns could also reflect the antigenic diversity of these viruses. In order to eliminate this possibility, we compared RNAs from viruses that are antigenically identical but differ in transforming ability.

The RNAs of avian sarcoma virus B77 and of its nontransforming derivatives NT B77: B77 and NT B77 are related viruses which were found to differ only in their ability to transform chick-embryo fibroblasts but are otherwise indistin-

guishable.[15]   A comparison of these two viruses should therefore reveal possible transformation-related differences between their RNAs.   Coelectrophoresis of native and heat-dissociated RNAs obtained from B77 and NT B77 clone 1 and 5 did indeed show such differences (Fig. 3).   The native RNAs of the nontrans-
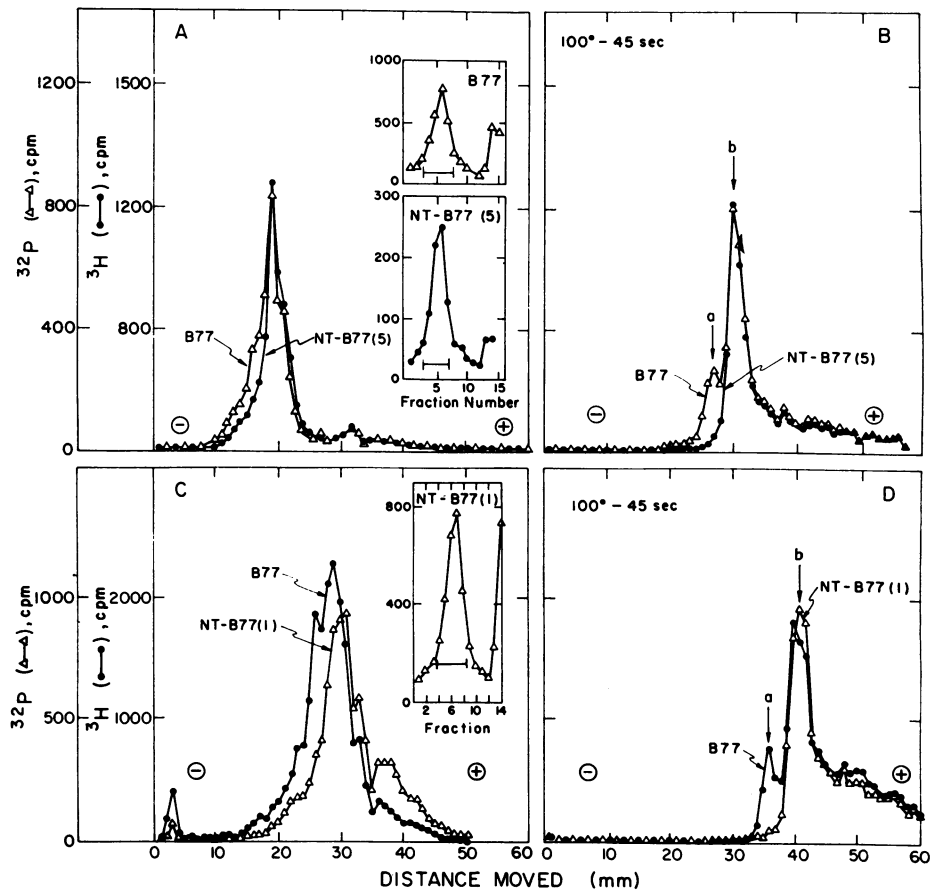

FIG. 3.   Coelectrophoresis of the 60–70S RNAs of B77 with those of two nontransforming derivatives, NT-B77 clone 5 and NT-B77 clone 1 before (*A,C*) and after (*B,D*) heat dissociation.   Conditions of electrophoresis and preparation of the 60–70S RNAs were as described in Figs. 1 and 2.

forming viruses migrated slightly faster than the RNA of B77.   In addition, electropherograms of the heat-dissociated RNAs demonstrated the absence of class *a* from NT B77 clone 5 (Fig. 3*B*) and from NT B77 clone 1 (Fig. 3*D*).   By contrast, both RNA classes, *a* and *b*, were present in transforming B77.   The same differences were seen when two other independent isolates of NT B77 (clones 3 and 4) were compared to B77.

Discussion.   The subunits obtained from avian sarcoma virus RNA by heating can be resolved into at least two classes of electrophoretic components, *a* and *b*.   All avian sarcoma viruses studied contained both classes of RNA subunits.

By contrast the avian leukosis viruses AMV, CZAV, and MAV-B, and four non-transforming derivatives of the sarcoma virus B77, lacked class *a* subunits.

The different electrophoretic mobilities of the RNA subunit classes *a* and *b* indicate that these segments of the viral RNA have different molecular weights[23] and suggest that they differ in their genetic information. However, gene duplications (and hence, repeats of the same information) in subunits *a* and *b* cannot be ruled out, although complete diploidy or triploidy of avian RNA tumor viruses is rendered unlikely by radiological data.[3,24] It is still uncertain whether the two RNA subunit classes of a given sarcoma virus are homogeneous themselves or whether they in turn encompass different RNA molecules which may be separable by more refined techniques.

The absence of class *a* RNA subunits from leukosis viruses and from various NT B77 clones may be related to the inability of these viruses to transform efficiently chick embryo fibroblasts in tissue culture. This idea is suggested by a comparison of B77 and NT B77 in which the qualitative differences in RNA structure correspond to a single biological difference, namely ability or inability to transform. Thus, it is tempting to speculate that only class *a* RNA contains genetic information required for transformation of fibroblasts. It is possible, however, that the presence of class *a* RNA is a consequence of the transformed state of the cell rather than its cause.

Class *a* RNA probably has a higher molecular weight than class *b* RNA[23]. Therefore, the absence of class *a* RNA in nontransforming viruses, may explain why the 60–70S RNAs of nontransforming viruses have higher electrophoretic mobilities than those of transforming viruses, such as SR RSV-A, which contain a large fraction of class *a* RNA.

The avian sarcoma virus strains described here differ from each other in the relative concentrations of the two RNA subunit classes. These quantitative differences reflect the average RNA composition in large populations of virus particles and leave open the question of whether individual particles show uniformity or variation with respect to their RNA complement. However, if one takes into account that the 60–70S RNA consists of approximately three subunits then it appears likely that populations of sarcoma viruses are heterogeneous with respect to their RNA structure. Such heterogeneity of a given virus strain is compatible with the rather broad and asymmetric distribution of 60–70S tumor virus RNAs in sucrose gradients[6] and polyacrylamide gels (Figs. 1–3). It may derive from the linkage of several RNA subunits including the minor RNAs obtained after heat dissociation of 60–70S RNA. The individual 60–70S RNAs may include subunits of either the same or different classes. A preponderance of class *b* subunits in avian sarcoma virus strains may therefore indicate the presence of virions lacking class *a* RNA. Such particles might also fail to induce transformation of fibroblasts. Preparations of RSV(O), in which the predominance of class *b* RNA subunits is particularly striking, have recently been shown to contain a nontransforming agent.[26] The presence of this agent could decrease the ratio of class *a* to class *b* subunits. This might also explain the failure to detect (presumably) class *a* RNA subunits in a previous study of the RNA of the Bryan RSV,[6] which contains a large excess of nontransforming

RAV.[25] Similar nontransforming virus may be present in other sarcoma virus strains, where they may have arisen spontaneously as segregants during the growth of virus stocks. Further work will be necessary to test these speculations.

Abbreviations: Viral strains; B77, avian sarcoma B77; NT, nontransforming (i.e. NT B77); Rous sarcoma, RSV; Schmidt-Ruppin, SR RSV-A; Prague, PR RSV-C; RSV of type O, RSV(O); avian myeloblastosis, AMV; myeloblastosis-associated, MAV-B; Carr Zilber-associated, CZAV.

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