Genetic Structure and Transforming Sequence of Avian Sarcoma Virus UR2

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We have recently shown that a newly isolated avian sarcoma virus, UR2, is defective in replication and contains no sequences homologous to the *src* gene of Rous sarcoma virus. In this study, we analyzed the genetic structure and transforming sequence of UR2 by oligonucleotide fingerprinting. The sizes of the genomic RNAs of UR2 and its associated helper virus, UR2AV, were determined to be 24S and 35S, respectively, by sucrose gradient sedimentation. The molecular weight of the 24S UR2 genomic RNA was estimated to be 1.1×10^6 , corresponding to 3,300 nucleotides, by gel electrophoresis under the native and denatured conditions. RNase T₁ oligonucleotide mapping indicated that UR2 RNA contains seven unique oligonucleotides in the middle of the genome and shares eight 5'- and six 3'-terminal oligonucleotides with UR2AV RNA. From these data, we estimated that UR2 RNA contains a unique sequence of about 1.2 kilobases in the middle of the genome, and contains 1.4 and 0.7 kilobases of sequences shared with UR2AV RNA at the 5' and 3' ends, respectively. Partial sequence analysis of the UR2-specific oligonucleotides by RNase A digestion revealed that there are no homologous counterparts to these oligonucleotides in the RNAs of other avian sarcoma and acute leukemia viruses studied to date. UR2-transformed non-virus-producing cells contain a single 24S viral RNA which is most likely the message coding for the transforming protein of UR2. On the basis of the uniqueness of the transforming sequence, we concluded that UR2 is a new member of the defective avian sarcoma viruses.

A unique feature of acute transforming retroviruses is the presence of a transforming gene in their genomes. In nondefective avian sarcoma viruses (ASVs), such as Rous sarcoma virus (RSV), the sarcomagenic sequence, known as *src*, is located between the envelope glycoprotein gene (*env*) and the 3' noncoding region, known as *c* (29), of the genome. Recent studies of a number of defective ASVs revealed that they contain transforming genes totally unrelated to *src*, and the location of these transforming genes within the genome is quite different from that of nondefective RSV (12, 16, 17, 22, 26, 37). Of several independent isolates of ASV recently characterized, Fujinami sarcoma virus (FSV) and PRCII appear to contain transforming sequences highly related to each other (26). This sequence is designated *fps* to denote that it is shared by these two viruses. The unique sequences of ASV Y73 (16) and of Esh sarcoma virus described more recently (9) were found to belong to yet another class, called *yes* (37). The transforming sequences of these two classes of ASVs are located at the middle of the genome and are both flanked by a portion of the group-specific antigen gene (*gag*) at the 5' end and by

the *c* region at the 3' end (12, 17, 37). Despite the unrelatedness of nucleotide sequences among *src*, *fps*, and *yes*, all of them code for proteins that are associated with a protein kinase activity (8, 9, 16, 24) which is considered to play a role in the process of cell transformation by these retroviruses.

We have recently described the isolation of two strains of ASV derived from spontaneous chicken fibrosarcomas (2). Initial studies of these two ASVs, designated UR1 and UR2, revealed that they are defective in replication and contain unique sequences unrelated to the *src* gene of RSV (2). Further study of UR1 showed that it contains a transforming sequence very closely related to that of FSV (33). The genome of UR1 codes for a polypeptide of 150 kilodaltons (p150) containing a portion of the *gag* protein. The p150 of UR1 is associated with a protein kinase activity whose properties are very similar to those of the transforming protein of FSV (33). We concluded that UR1 is a member of the FSV-related defective ASVs (33).

In this paper, we describe the genetic structure and transforming sequence of UR2 analyzed by RNase T₁ oligonucleotide fingerprint-

ing. The genome of UR2 is about 3.3 kilobases (kb) and contains a relatively long 5' *gag* sequence and a relatively short unique sequence as compared with other defective ASVs. The transforming sequence of UR2 is unrelated to those of other ASVs and avian acute leukemia viruses studied so far. Thus, UR2 represents a new class of ASV.

MATERIALS AND METHODS

Cells and viruses. Preparation and maintenance of chicken embryo fibroblast cultures and propagation of viruses have been described (10). ASV UR2 has been described recently (2). A helper virus associated with UR2, UR2-associated virus (UR2AV), was isolated from a terminal dilution of the UR2 stock. The virus belongs to subgroup A as described previously (2).

UR2-transformed nonproducer cell cultures were prepared by the methods published earlier (11). Briefly, a stock of UR2(UR2AV) was serially diluted and used to infect chicken embryo fibroblasts. Serum against Schmidt-Ruppin RSV of subgroup A was included in the agar medium used to overlay the infected cell cultures to prevent the spread of UR2AV, which was found to be antigenically related to Schmidt-Ruppin RSV of subgroup A. Individual foci from dishes containing fewer than 10 foci were isolated and plated on uninfected chick embryo fibroblasts. Of 23 cultures derived from these foci, 10 were nonproducers, as judged from lack of reverse transcriptase activity and production of infectious virus. All nonproducers released infectious UR2 upon superinfection with Rous-associated viruses (RAV-1 or RAV-2).

Preparation of cellular and viral RNAs. Isotope-labeled chicken cellular and viral RNAs were prepared by procedures described previously (30–32).

Analysis of the size of viral RNAs. Separation of RNAs in 2.2% sodium dodecyl sulfate (SDS)-polyacrylamide gels and 1% agarose slab gels followed published procedures (6, 21). Elution of RNA from polyacrylamide gels was described recently (33). Velocity sedimentation was performed with 10 to 25% linear sucrose gradients containing 0.01 M Tris-hydrochloride (pH 7.2), 0.1 M NaCl, 1 mM EDTA, and 0.1% SDS. Viral and marker RNAs were mixed, heat denatured, and sedimented in the sucrose gradients at 40,000 rpm for 4.5 h at 22°C in an SW40 rotor.

Preparation of cDNAss and dC-tailed cDNAss. Preparation of DNA complementary to the 5'-terminal viral RNA sequence, called "strong-stop" cDNA (cDNAss), essentially followed published procedures (13, 15). The procedure for preparing ³²P-labeled cDNAss of high specific activity for filter hybridization was described recently (33). The procedures for making a large quantity of cDNAss of low specific activity were essentially the same, except that about three times more purified virus and 40 times less [³²P]dCTP were present in the reaction mixture. From 57 absorbancy (260 nm) units of purified RAV-2 virions, about 0.9 µg of cDNAss with specific activity of 2.1×10^5 cpm per µg was obtained. Deoxycytidylic acid (dC) residues were added to the 3' end of cDNAss with polynucleotide terminal transferase. The reaction mixture of 50 µl contained 0.1 M potassium cacodylate (pH 7.2), 1 mM CoCl₂, 1 mM dithiothreitol, 3 mM

dCTP, 16 µg of bovine serum albumin, 100 U of terminal transferase, and appropriate amounts (up to 5 µg) of single- or double-stranded DNA to be extended. The reaction was carried out at 37°C for 3 h. The enzyme was removed by phenol and chloroform extractions, and the dC-extended DNA was concentrated by ethanol precipitation. The dC-tailed cDNAss was selected by fractionation on an oligodeoxyguanylic acid [oligo(dG)]-cellulose column as follows. The cDNA was suspended in 0.5 ml of buffer containing 10 mM EDTA, 0.01 M Tris-hydrochloride (pH 7.2), and 0.2% SDS (ETS) and was heat denatured at 100°C for 1 min; 10 M LiCl was then added to a final concentration of 0.5 M. A 0.5-ml oligo(dG)-cellulose column was washed with 2 ml of ETS (400 µg of yeast RNA was included in the buffer when the column was first packed) and equilibrated with ETS containing 0.5 M LiCl (0.5 M LETS) before use. The sample was applied to the column and subsequently washed with 2-ml volumes of ETS containing 0.5 M LiCl (0.5 M LETS), ETS containing 0.1 M LiCl (0.1 M LETS), ETS containing 10% formamide, and ETS containing 90% formamide. Fractions of 0.5 ml were collected, and the cDNAss was monitored by Cerenkov radiation. Oligo(dC)- and poly(dC)-tailed cDNAss was usually eluted with 10% and 90% formamide buffers. cDNA from these fractions was pooled and precipitated with ethanol. All solutions for the oligo(dG) column were precooled and kept in ice during the fractionation.

Isolation of 24S UR2 RNA. UR2 RNA was purified by the following procedures. The ³²P-labeled 50 to 70S RNA complex of UR2 and UR2AV was heat denatured and fractionated on an oligodeoxythymidylic acid-cellulose column. The polyadenylic acid [poly-(A)]-containing RNAs were separated either on a sucrose gradient or by SDS-polyacrylamide gel electrophoresis as described above. The 24S RNA was recovered from either the gradient or the gel. This procedure achieved only a partial purification of UR2 RNA, and the preparation was still contaminated by various amounts of degraded UR2AV RNA derived from the 3' half of the molecule. The gel- or gradient-purified 24S RNA was then hybridized with the cDNAss extended with dC residues. Hybridization was carried out in the 50 µl of reaction solution containing 5 mM Tris-hydrochloride (pH 7.2), 3× SSC (SSC: 0.15 M NaCl plus 0.015 M sodium citrate), 3 mM EDTA, 0.03% SDS, 30% formamide, and appropriate amounts of partially purified 24S RNA and dC-tailed cDNAss. The reaction took place in a sealed capillary at 50°C for 3 h, after initial denaturation at 95°C for 1 min. After hybridization, the RNA and DNA mixture was precipitated with ethanol and dissolved in 10 µl of buffer containing 0.01 M Tris-hydrochloride (pH 7.2), 0.01 M NaCl, 1 mM EDTA, and 0.1% SDS. Then 0.5 ml of 0.5 M LETS was added to the RNA-DNA solution. The sample was applied directly to the oligo(dG)-cellulose column without heat denaturation and then fractionated as described above, except that a double volume of 0.1 M LETS was used for washing before elution with 10% and 90% formamide. The purified 24S UR2 RNA was then subjected to RNase T₁ fingerprinting analysis.

RNase T₁ fingerprinting and oligonucleotide mapping. The details of the method have been described (31, 34). A ³²P-labeled 50 to 70S RNA mixture of UR2

and UR2AV (or of purified UR2AV alone), gel-purified 35S UR2AV, and cDNAss-selected 24S UR2 RNA were analyzed and compared to determine the patterns of T_1 oligonucleotides for the UR2 and UR2AV genomic RNAs (Fig. 2 and other data not shown). An oligonucleotide map of UR2AV was prepared by using both gel-purified 35S RNA and RNA obtained from purified UR2AV. Because the amount of cDNAss-selected 24S RNA was limited, the oligonucleotide map of UR2 RNA was constructed by using partially purified 24S RNA from polyacrylamide gels.

Analysis of intracellular viral RNAs. Isolation of poly(A)-containing RNAs from infected cells, separation of the RNAs on agarose gel, transfer to nitrocellulose paper, and hybridization with [^{32} P]cDNAss have been described (28, 33).

RESULTS

Size of UR2 genome. The radiolabeled 50 to 70S RNA mixture extracted from purified UR2 and UR2AV virions was heat denatured and fractionated in a 2.2% SDS-polyacrylamide gel. Two RNA components were resolved: an RNA species coelectrophoresed with RAV-2 35S RNA, and a smaller component migrated between the 18S and 28S marker RNAs (Fig. 1A). The 50 to 70S RNA of pure UR2AV obtained by terminal dilution of a stock of UR2(UR2AV) was similarly analyzed. Figure 1B shows that UR2AV contains only a single RNA component which has a size similar to that of RAV-2. Therefore, the smaller RNA present in the mixture of UR2 and UR2AV RNAs is most likely the genome of UR2. The size of the smaller RNA species eluted from a gel similar to that shown in Fig. 1A was further determined by velocity sedimentation in a sucrose gradient. The small RNA sedimented between 18S and 28S chicken ribosomal RNAs and was estimated to have a sedimentation coefficient of 24S (Fig. 1C). On the basis of the mobility of each RNA species under denatured conditions in an agarose gel (18–20) with ^{32} P-labeled single-stranded λ Hind III restriction fragments used as markers, the molecular weights of the 24S UR2 and 35S UR2AV RNAs were determined to be about 1.1×10^6 and 2.5×10^6 , respectively, corresponding to 3,300 and 7,500 nucleotides (Fig. 1D).

Analysis of the UR2 unique sequence. The size analysis of UR2 and UR2AV suggests that the 24S RNA is most likely the genome of UR2 containing the sarcomagenic information, since purified UR2AV is not able to transform cells. To determine whether the 24S RNA indeed contained the transforming gene, we compared sequences of 35S and 24S RNAs by RNase T_1 oligonucleotide fingerprinting. The ^{32} P-labeled 50 to 70S RNA mixture of UR2 and UR2AV was heat denatured and passed through an oligodeoxythymidylic acid-cellulose column, to re-

move degraded non-poly(A)-containing RNAs. The resulting poly(A)-containing RNAs of UR2 and UR2AV were fractionated in a preparative polyacrylamide gel similar to that shown in Fig. 1A. The 35S and 24S eluted from such a gel were analyzed by RNase T_1 fingerprinting. The fingerprint pattern of the 35S RNA (Fig. 2C) isolated from the gel is identical to that of 50 to 70S RNA of UR2AV (Fig. 2D) purified by terminal dilution. This result confirmed that the 35S RNA is the genome of UR2AV. Unlike the 35S RNA which could be obtained in a virtually pure form by the procedures described above, the fingerprint pattern of gel-purified 24S RNA revealed that it was always contaminated with a significant amount of poly(A)-containing RNA fragments derived from the 3' half of the UR2AV genome (data not shown). This phenomenon was most likely due to a large excess of UR2AV over UR2 in the virus preparations obtained from the infected cultures. Repeated efforts to improve the ratio of UR2 to UR2AV by preparing cultures with foci selected from plates infected with UR2(UR2AV) did not totally overcome the problem. This complication made it difficult to assess the sequences common to 24S and 35S RNAs, particularly within the 3' half of the genome. To obtain pure 24S RNA, we undertook a further step of purification by selecting for RNAs containing an intact 5'-terminal sequence in addition to selecting by size and presence of 3' poly(A) sequences. cDNAss complementary to the 5'-terminal 101 nucleotides of RAV-2 RNA was prepared. This cDNAss was extended at the 3' end with dC residues. The dC-tailed cDNAss was hybridized with 24S RNA eluted from a preparative gel. The mixture of hybrid and free RNA was fractionated in an oligo(dG)-cellulose column to select for RNA that was able to hybridize with dC-tailed cDNAss. The 24S RNA recovered from the oligo(dG) column was then analyzed by RNase T_1 fingerprinting.

Figure 2B shows that 19 large RNase T_1 -resistant oligonucleotides were generated from purified 24S UR2 RNA, whereas 39 spots were generated from 35S helper virus RNA (Fig. 2C and D). These data are consistent with the complexity of the respective RNAs, as judged from their sizes, and indicate that the 24S RNA obtained by this method is rather pure. Comparison of the fingerprints of 35S and 24S RNAs showed that the 24S RNA contained 7 unique oligonucleotides (no. 61 to 66) not present in UR2AV RNA and shared 11 oligonucleotides (the rest of the numbered spots in Fig. 2B) with UR2AV RNA. Moreover, 5 to 11S poly(A)-containing RNA fragments (up to about 700 nucleotides) from both species of RNA generated identical fingerprint patterns and shared 4

FIG. 1. Analysis of the size of UR2 and UR2AV genomic RNAs. (A and B) Polyacrylamide gel electrophoresis. ^{32}P -labeled 50 to 70S RNA mixture from purified UR2 and UR2AV virions (A) was mixed with ^3H -labeled marker RNAs, heat denatured, and electrophoresed in a 2.2% SDS-polyacrylamide gel. ^3H -labeled 50 to 70S RNA complex of biologically purified UR2AV was similarly analyzed with ^{14}C -labeled RAV-2 35S RNA (B). (C) Sucrose gradient sedimentation. The ^{32}P -labeled smaller RNA component of the UR2 and UR2AV RNA mixture was eluted from a polyacrylamide gel similar to that shown in A and further analyzed by velocity sedimentation in a 10 to 25% linear sucrose gradient. ^3H -labeled chicken cellular RNAs were included as internal markers. (D) Agarose gel electrophoresis. Gradient-purified unlabeled 24S UR2 (d) and 35S UR2AV RNAs (c) were denatured with 1 M glyoxal in the presence of 50% dimethyl sulfoxide at 50°C for 30 min. The denatured RNAs were electrophoresed in 1% agarose slab gel. ^{32}P -labeled chicken 18S and 28S ribosomal RNAs (b) and heat-denatured λ Hind III restriction fragments were used as the molecular-weight markers. The RNAs and DNA markers were transferred to nitrocellulose paper (Materials and Methods) and hybridized with [^{32}P]cDNA made from UR2 24S RNA. The numbers at the left indicate the molecular weights in megadaltons of single-stranded λ Hind III restriction fragments. In a separate experiment, ^{32}P -labeled 50 to 70S RNA of UR2 and UR2AV was heat-denatured and fractionated in an oligodeoxythymidylic acid-cellulose column. The poly(A)-containing UR2 and UR2AV RNAs (e) were concentrated and analyzed in 1% agarose gel as described above. Lane f shows ^{32}P -labeled 35S RAV-2 RNA and rRNA markers analyzed in parallel.

intermediate-sized oligonucleotides, 38, 39, 40, and C' (Fig. 2A and E). The results of the partial sequence analysis of the oligonucleotides from UR2 and UR2AV RNAs are presented in Table 1. The oligonucleotide map of UR2AV RNA was prepared to locate the spots shared with UR2 RNA (Fig. 2 and 3). Eight shared oligonucleotides, 20, 9, 7a, 30, 42, 10b, 19, and cap, are

located at the 5' end of the UR2AV genome, since they are absent or only partially recovered in 23 to 32S poly(A)-containing UR2AV RNAs (Fig. 2G) and are the only spots still missing in that RNA fraction in comparison with the 35S genomic RNA. Two of them, 7a and cap, are present in several strains of avian retroviruses (Fig. 3). Two shared oligonucleotides, 11 and

12a, in addition to the spots, 38, 39, 40 and C', in the *c* region mentioned above, are located in the 3' region of UR2AV RNA. Both 11 and 12a are highly conserved *env*-specific spots. These two oligonucleotides, 11 and 12a, are located at 96 and 594 nucleotides, respectively, upstream from the termination codon of gp37 in the RNA of Schmidt-Ruppin RSV of subgroup A (T. Takeya and H. Hanafusa, unpublished data). Therefore, UR2 RNA contains at least some gp37 *env* sequences in common with those of its helper virus RNA. The ordering of oligonucleotides in UR2 RNA was done with the partially purified UR2 24S RNA obtained from a polyacrylamide gel (data not shown). By excluding the contaminating oligonucleotides derived from the 3' half of helper virus RNA, i.e., those which were not present in purified 24S UR2 RNA (Fig. 2B), an oligonucleotide map of UR2 could be constructed (Fig. 3). The seven UR2-specific

oligonucleotides are unique in their base composition and are not found in either the avian sarcoma or acute leukemia viruses studied so far (3, 5, 7, 12, 33, 34, 37). This is consistent with the result of nucleic acid hybridization studies carried out with UR2 RNA and the cDNA's specific for the transforming sequences of various ASVs and acute leukemia viruses (M. Shibuya, H. Hanafusa, and P. C. Balduzzi, J. Virol., in press). We conclude that UR2 contains a unique transforming sequence, and thus it represents a new class of ASV.

mRNA in UR2-infected cells. Some of the avian acute transforming retroviruses form spliced subgenomic mRNA's (1, 4, 14, 25, 35), whereas others, such as UR1 and FSV, give rise to only mRNA's of genomic size (33; M. Shibuya and H. Hanafusa, unpublished data). To see whether UR2 produces subgenomic mRNA's, we have analyzed viral RNAs present

FIG. 2. Fingerprint patterns of RNase T₁-resistant oligonucleotides of UR2 and UR2AV RNAs. A: 5S to 10S poly(A)-containing RNA prepared from partially purified 24S UR2 RNA obtained from a polyacrylamide gel. B: cDNass-purified 24S UR2 RNA. C: 35S UR2AV RNA purified from the 50 to 70S complex of UR2 and UR2AV RNAs in a polyacrylamide gel. D: 50 to 70S RNA of UR2AV purified by terminal dilution. E, F, G: 5 to 11S, 12 to 22S, and 23 to 32S poly(A)-containing RNAs prepared from gel-purified 35S UR2AV RNA similar to that described in C. H: tracing of the fingerprint in D. The spots indicated by arrows in D and darkened in H are those shared by UR2 and UR2AV RNAs.

TABLE 1. RNase T₁-resistant oligonucleotides of UR2 and UR2AV

Spot no.	RNase A digestion products ^a
UR2-specific	
61	4U, 4C, G, AC, AU, AAC, AAU
62	7U, 2C, 2AC, 2AU, AG
63	2U, 4C, AC, AU, AG, AAC
64	3U, C, G, AC, AU, A ₃ C, A ₄ N
65	4U, 2C, G, 2AC, AU, AAU
66ab	7U, 7C, G, 2AC, AU, AAU, AAG
UR2AV	
1	5U, 8C, 2AC, 2AU, AG, 2AAC, AAU
2b	4U, 8C, 2AC, 2AU, AAC, AAG
3	C, G, 2AC, A ₃ U, A ₄ N
4	2U, 3C, AC, AAU, A ₃ C, A ₄ G
5	5U, 5C, G, 2AC, 4AU
6	5U, 8C, G, 2AC, 2AAU
7a	5U, 6C, AAG, A ₄ N
8	3U, 7C, G, 2AC, AU
9	4U, 9C, G, AC, 2AU
10ab	10U, 12C, 3AC, AU, AG, A ₅ N, A ₅ G
11	2U, 3C, G, 2AC, AU, AAC
12a	4C, 2AC, AU, AG, AAC
12b	4C, G, 2AC, A ₄ N
12c	U, 4C, 3AC, AU, A ₄ G
14	U, 5C, 3AC, AAG
15	3C, AU, AG, A ₃ N
16	5U, 3C, G, 2AU, A ₃ C
17	2U, 2C, G, 3AU, 2AAU, A ₃ C
18	8U, 6C, G, AU
19	U, 4C, G, 2AC, AU, AAC
20	4U, 3C, G, 2AC, 2AU
21	4U, 2C, G, 2AC, 2AU, AAU
22	U, 2C, G, 2AU, AAU, A ₃ U
23	4U, 4C, G, AC, AU
24	4U, 3C, AC, AG, AAC
26	5U, 3C, G, AU, AAU
27	3U, C, G, 3AU, AAU
28	5U, 3C, G, 2AU
29	6U, 2C, 2AU, AG
30	2U, 2C, G, AC, AAC, A ₃ C
31	2U, 4C, G, AC, AU, AAC
32a	4C, G, 4AC
33	5C, 2AC, AG
34	4C, AC, AG, AAC
35ab	6C, 2AC, 2AG, AAC, A ₄ N
38	2U, 2C, G, AC, AU, AAU
39	3U, 3C, 2AU, AG
40	2U, 2C, G, AC, AU, AAU
41	U, 2C, AC, AG, AAU
42	U, 4C, G, AC, AAC, A ₃ C
C ^b	2U, 2C, AU, AG, A ₃ C
cap ^c	m ⁷ G ^{5'} ppp ^{5'} GmpCp (5U, 6C, 3AC, 2AU)G

^a Oligonucleotides were recovered from fingerprints of either total viral RNA or poly(A)-containing RNA fragments and analyzed by the procedure described before (31, 32).

^b This oligonucleotide has a composition of RNase A-resistant fragments quite distinct from that of oligonucleotides C widely present in other avian retroviruses (29); apparently, they do not represent the homologous sequences.

^c The RNase A digestion product of this cap oligo-

in infected cells. Total poly(A)-containing RNAs from chicken cells infected with UR2(UR2AV) and from UR2-infected nonproducer cells were separated by agarose gel electrophoresis after denaturation with glyoxal and then transferred to nitrocellulose paper. Virus-specific RNAs were detected by hybridization with [³²P]cDNAs synthesized from purified UR2 and UR2AV virions. Three species of RNAs were detected in UR2(UR2AV)-infected cells (Fig. 4B). The 35S RNA and the fastest moving 22S RNA are apparently the helper genomic RNA and its spliced *env* mRNA, respectively, since they are absent in UR2-transformed nonproducer cells (Fig. 4C and D). The third species of RNA migrated at a position corresponding to the size of the UR2 genome, i.e., 24S (see also Fig. 1). The intensity of the 24S band in clone 12 is much less than that of clone 15. This could be due to fewer transformed cells in clone 12 culture, or it could be due to the decreased expression of 24S RNA in that culture. Since both clones of UR2-transformed nonproducer cells displayed only a single species of intracellular viral RNA of genomic size (Fig. 4C and D), we conclude that UR2-infected cells do not contain viral mRNA's detectably different in size from the genomic RNA. Therefore, the 24S UR2 RNA detected in both producer and nonproducer cells is most likely the message for the transforming protein of UR2.

DISCUSSION

UR2 was isolated from a spontaneous chicken sarcoma and has a sarcomagenic potential similar to that of RSV (2). Our data indicate that the overall genetic structure of UR2 is different from that of RSV but is similar to that of several other defective ASVs studied recently (9, 12, 17, 33, 37). However, the genome of UR2 contains a unique sequence unrelated to any of the known ASVs. Direct evidence that this sequence is the transforming gene of UR2 is not available yet. Nevertheless, the fact that this is the only sequence that is not shared between UR2 and UR2AV, an agent which does not induce cell transformation, strongly suggests that it contains at least part of the transforming gene. Moreover, the UR2 genome codes for a unique protein of 68,000 daltons, associated with a protein kinase activity (R. A. Feldman, L.-H. Wang, H. Hanafusa, and P. C. Balduzzi, J. Virol., in press). Because of its uniqueness this sequence is designated with a new name, *ros*. UR2 therefore represents a fourth class of ASV, the other three being represented by RSV, FSV and Y73. Each class pos-

nucleotide is identical to that of class II cap oligonucleotide described previously (32).

FIG. 3. Oligonucleotide maps of UR2 and UR2AV RNAs. The scale on the left represents the UR2AV genome and is measured in kilobases (kb) from the 3' end of the RNA molecule. The gene order and the approximate range of each gene, including the 3' "common" region c (29) is shown. The circled oligonucleotides are those that are highly conserved among avian retroviruses (29). The order of spots within each set of parenthesis is not certain. Oligonucleotides within the bracket are UR2 specific.

sesses a unique transforming sequence which has a counterpart in normal chicken cells (27, 36; Shibuya et al., in press).

UR2 has a genome of about 3.3 kb, which is the smallest among the known avian retroviruses. Interestingly, the genome of UR2 shares a relatively large number of oligonucleotides at the 5' end with its helper virus RNA. Since poly(A)-tagged RNA fragments up to 700 nucleotides from UR2 and UR2AV had identical fingerprint patterns (Fig. 2), we estimated that they shared about 0.7 kb at the 3' ends of their

genomes. The remaining 2.6 kb of the UR2 genome is composed of two domains of sequences: the UR2-specific sequence which contains 7 large oligonucleotides ranging from 14 to 20 nucleotides in length and the 5' shared partial *gag* sequences which contains 8 large oligonucleotides ranging from 14 to 25 nucleotides in length (Table 1). We therefore suggest that the two domains of sequence have about equal complexity and estimated 1.2 and 1.4 kb for the unique and 5' shared regions, respectively. The genome of UR2 encodes a *gag*-related polypeptide of 68,000 daltons (P68), which can account for about 80% of the coding capacity of the joined *gag-ros* sequences. P68 appears to be the only product of the UR2 genome since in vitro translation of 24S UR2 RNA generates polypeptides similar to P68 in size (Feldman et al., in press). In addition, there is apparently no sub-genomic mRNA in UR2-infected cells (Fig. 4). On the basis of serological and peptide analyses, it has been suggested that P90, P140, and P105 encoded by Y73, FSV, and PRCII, respectively, contain only the *gag*-coded protein p19 (9, 23, 37). It does appear, therefore, that a large por-

FIG. 4. Viral RNAs present in UR2- and UR2AV-infected cells and UR2-infected non-virus-producing cells. About 8 μ g of poly(A)-containing RNAs isolated from UR2- and UR2AV-infected cells (B) or from UR2-infected non-virus-producing cell clones, no. 12 (C) and 15 (D), were separated in an agarose gel after denaturation of RNAs with glyoxal. The RNAs in the gel were transferred to nitrocellulose paper and hybridized with [^{32} P]cDNAs prepared from UR2 and UR2AV virions. Lane A shows ^{32} P-labeled marker RNAs analyzed in parallel.

tion of these polyproteins must be coded for by the respective unique sequences. By contrast, a substantial portion of the UR2-specific protein P68 is probably derived from the *gag* sequence since we estimated that about 1.4 kb of 5' UR2AV RNA sequence is present in the UR2 genome. It will be interesting to determine precisely how much *gag* information is present in P68 and which portion of the unique sequence is responsible for the kinase activity associated with it. The direct sequencing of the UR2 genome will be necessary to answer this question.

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