JOURNAL OF VIROLOGY, Feb. 1987, p. 344–353
0022-538X/87/020344-10$02.00/0

Vol. 61, No. 2

Transcriptional Activities of Mammalian Genomes at Sites of Recombination with Foreign DNA

MANFRED SCHULZ, UTA FREISEM-RABIEN, ROLF JESSBERGER, AND WALTER DOERFLER*

Institute of Genetics, University of Cologne, Cologne, Federal Republic of Germany

Received 16 July 1986/Accepted 7 October 1986

The nucleotide sequences of several sites of recombination between adenovirus DNA and hamster, mouse, or human cell DNAs were determined. These sites of recombination had been cloned from adenovirus type 2 (Ad2)- or type 12 (Ad12)-transformed cells, from Ad12-induced tumor cells, or from a symmetric recombinant between Ad12 DNA and human cell DNA. One important precondition for the generation of recombinants between host and foreign DNAs might be the establishment of a chromatin configuration that permits access of foreign DNA and of the recombination machinery to cellular DNA. Such favorable chromatin structures might arise during cellular DNA replication or transcription or both. As a first approach toward investigating these more complex problems of foreign DNA insertion, we determined transcriptional activities of cellular DNA sequences at viral junction sites. The sites of linkage investigated in this study with respect to their transcriptional activities were those previously cloned and sequenced (W. Doerfler, R. Gahlmann, S. Stabel, R. Deuring, U. Lichtenberg, M. Schulz, D. Eick, and R. Leisten, Curr. Top. Microbiol. Immunol. 109:193–228, 1983). In addition, a site from cell line HA12/7 which is described in this paper was also analyzed. The results presented demonstrate that the cellular DNA sequences involved in linkage to viral DNA at five completely different sites in DNA from three different species are transcribed into RNAs even in cells which have not been transformed or infected by adenovirus. Some of these RNAs were cytoplasmic and were not poly(A)$^+$. Human cell DNA sequences at the junction to Ad12 DNA in SYREC2 DNA were transcribed into poly(A)$^+$ cytoplasmic RNA which could be translated in vitro. These results are consistent with the notion that at least some of the cellular DNA sequences at sites of insertion of adenovirus (foreign) DNA are transcriptionally active and thus provide an opportunity for recombination.

The mechanism of recombination in mammalian cells is not yet well understood. The efficiency of recombination in mammalian cells appears to be considerable, as evidenced by the ready uptake of any foreign DNAs, e.g., of viral origin, and their stable integration into the host chromosome. From several studies, there is evidence that recombination processes can involve either homologous (32, 38) or heterologous (38, 43, 44) recognition between cellular and foreign nucleotide sequences. Studies on the mechanism of recombination in mammalian cells will require both structural analyses of the nucleotide sequences at the sites of genetic exchange and work on the enzymes and other factors catalyzing the reaction. Available technology has allowed nucleotide sequence studies to be advanced the furthest.

Since adenovirus-transformed cell lines provide a well-characterized source of clonal derivatives of recombination events, a number of junctions between host and viral DNAs were analyzed in recent years in an attempt to evaluate the sequence requirements, if any existed, for recombination in mammalian cells. These results have been summarized previously (9). Although many structural features have been found, such as stemmed loops, patch homologies, and direct homologies between viral DNA and the cellular sequence replacing it, there was no evidence that any of these signals would be strictly required for these recombination events or could universally direct them (9, 29). Moreover, evidence could not be adduced that foreign (viral) DNA had been inserted predominantly at specific cellular DNA sequences. On the other hand, the termini of both the adenovirus type 2 (Ad2) and Ad12 DNA molecules were preferred sites of recombination with host DNA in most of the recombination sites investigated. Patch-type homologies in nucleotide sequences between viral and cellular DNAs were invoked in the stabilization of some of the recombination events (7, 9, 13, 34, 36).

Analyses of the unoccupied insertion sites corresponding to sites of integration or recombination from the Ad2-transformed hamster cell line HE5, the Ad12-induced mouse tumor CBA-12-1-T, the Ad-12 induced hamster tumor cell line CLAC1, the Ad12-transformed hamster cell line HA12/7, and the symmetric recombinant SYREC2 revealed transcriptional activities at all of these cellular sequences in hamster, mouse, and human cells. The finding of transcriptionally activated states at most of the hitherto analyzed sites of recombination with foreign (adenoviral) DNA in mammalian genomes prompted the hypothesis that transcriptional activity at these sites of recombination was a necessary but not sufficient precondition.

MATERIALS AND METHODS

Cells and virus. The following cell lines were propagated in culture in Dulbecco modified Eagle medium (3) containing 10% fetal bovine serum: human HeLa and KB cells, the B3 subline (37) of BHK21 hamster cells, mouse LM or Ltk$^-$ cells, and the Ad12-transformed hamster cell line HA12/7 (40).

Ad12 was replicated on monolayer or suspension cultures of human KB or HeLa cells and purified as described previously (8). The origin of the symmetric recombinant (SYREC2) between the left terminus of Ad12 DNA and human KB cell DNA was described previously (5, 6).

Cloned DNA fragments. Cloned junction sequences between Ad12 DNA and cellular DNA or cellular DNA from preinsertion sites corresponding to these junctions were

* Corresponding author.

isolated from the Ad12-induced mouse tumor CBA-12-1-T or mouse LM cells (31), from the Ad12-induced hamster tumor cell line CLAC1 (34) or hamster B3 cells, from the Ad12-transformed hamster cell line HA12/7 (40; R. Jessberger, S. Stabel, and W. Doerfler, manuscript in preparation), or from the symmetric recombinant SYREC2 (5), as described previously. For certain mapping experiments and for the determination of nucleotide sequences, specific DNA fragments were subcloned into bacteriophage M13 DNA (23). In particular, the M13-based vectors mp8, mp9, mp10, and mp11 were used. Standard subcloning procedures were used. Double-stranded M13 DNA was transfected into bacterial strains by using standard methods of bacterial transfection (24). In some experiments, cloned DNA fragments used as probes were excised from the vector DNA, isolated by agarose gel electrophoresis, and eluted by the DEAE-cellulose filter technique (10).

Extraction and purification of RNA. Methods for extraction and purification of RNA from mammalian cells were detailed previously (14, 21). In control experiments, purified preparations were treated with RNase-free DNase (Bethesda Research Laboratories, Inc.), or with RNase A to ascertain that bands in RNA transfer hybridization experiments actually represented RNA.

S1 analyses of RNAs of cellular origin. Previously published methods were used for S1 nuclease analyses of RNAs of cellular origin (4, 21). For hybridization of DNA fragments to RNA, temperatures between 50 and 60°C were used. The S1 nuclease was added at 200 U per assay.

Hybridization of RNA on Northern blots to ^{32}P-labeled cellular DNA fragments. RNA was size fractionated by electrophoresis on agarose gels containing 2.2 M formaldehyde. RNA samples were applied in 50% formamide–2.2 M formaldehyde–20 mM 3-(*N*-morpholino)propanesulfonic acid (MOPS)–5 mM sodium acetate (pH 7.0)–1 mM EDTA–1% Ficoll–bromophenol blue. After electrophoresis, RNA was transferred to nitrocellulose filters as described previously (1, 30). Filters were subsequently paper dried and were then kept at 80°C for 2 h. After a preincubation of at least 4 h at 42°C in 50% formamide–0.6 M NaCl–0.06 M sodium citrate–0.05 M sodium phosphate (pH 6.5)–0.1% Ficoll 400–0.1% polyvinylpyrrolidone–0.1% bovine serum albumin–500 μg of salmon sperm DNA per ml, the filters were incubated at 42°C in a fresh solution of the above composition but also containing 10% sodium dextran sulfate and containing only 100 μg of salmon sperm DNA per ml. This solution also contained the cloned cellular DNA fragment which had been ^{32}P-labeled by nick translation (27). About 0.5 to 1 μg of the labeled probe DNA, corresponding to 1×10^8 to 5×10^8 cpm, was added. These solutions were boiled for 10 min prior to use. After incubation for 16 to 24 h, the filters were washed three times for 10 min each at room temperature in 0.3 M NaCl–0.03 M sodium citrate–0.1% sodium dodecyl sulfate (SDS) and three times for 10 min each at 50°C in 15 mM NaCl–1.5 mM sodium citrate–0.1% SDS. The filters were dried and autoradiographed on Kodak XAR-5 film.

Hybrid selection of RNA. Published methods were used for hybrid selection of RNA (11, 26). Human cytoplasmic RNA was hybrid selected on the cloned 1.4-kilobase (kb) SYREC2 junction DNA fragment (5). In brief, plasmid DNA was sonicated and heat denatured, and 20 μg of this DNA was applied to nitrocellulose filters (3 × 3 mm) in several 3-μl portions. After addition of one 3-μl portion, the filters were dried before the next 3-μl portion was pipetted. Filters were dried overnight at room temperature, washed twice in 0.9 M NaCl–0.09 M sodium citrate, dried overnight again, treated at 80°C for 2 h, and kept at −20°C before use. Two filters loaded with DNA were incubated in an Eppendorf tube with 100 to 150 μg of human cytoplasmic RNA in 50% formamide–0.01 M piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (PIPES; pH 6.8)–0.4 M NaCl for 3 h at 50 to 60°C (cf. Fig. 8). Subsequently, filters were washed at 55°C in 0.15 M NaCl–15 mM sodium citrate–0.5% SDS and then three times in 2 mM EDTA. Finally, the filters were heated to 100°C in 0.3 ml of H_2O for 1.5 min to release the RNA. The RNA was immediately frozen in liquid N_2. Prior to in vitro translation, the RNA solution was slowly thawed, 25 μg of calf liver tRNA (Boehringer Mannheim Biochemicals) was added, and the RNA was ethanol precipitated.

Standard methods of molecular biology. The techniques of Southern blotting (33, 35), RNA blotting (1, 30), nucleic acid hybridization (41), nick translation of DNA probes (27), nucleotide sequence determination (22, 28), in vitro translation of RNAs in a cell-free reticulocyte lysate (11, 25), selection of poly(A)$^+$ RNA (2), and SDS-polyacrylamide gel electrophoresis on 10 or 15% gels (20) were used as described previously.

Computer analyses. For nucleotide sequence comparisons, programs of the University of Wisconsin Genetics Computer Group were used. The nucleotide sequences determined in this study or from previous analyses on the junction sites mentioned above were compared with data stored in the European Molecular Biology Laboratory Nucleotide Sequence Data Library, Heidelberg, Federal Republic of Germany, or the Genbank Data Library. A VAX/VMS computer (Digital Equipment Corp.) was used for these investigations.

RESULTS

Overview of transcriptional activities at junction sites between adenovirus and cellular DNAs. We analyzed cellular transcriptional activities at sites of linkage between viral and cellular DNAs in the Ad2-transformed hamster cell line HE5 and in the Ad12-induced mouse tumor CBA-12-1-T (14). In both instances, RNAs of about 300 and about 330 nucleotides in length, respectively, were detected with homologies to the cellular DNAs immediately abutting the sites of junction with adenovirus DNA. In cell line HE5 and in hamster cells of many derivations, the low-molecular-weight RNA occurred at low copy numbers in the cytoplasm of cells, the RNA was not poly(A)$^+$, and the sequence of homology exhibited no significant open reading frames. The RNA with homology to the mouse cellular sequence from the tumor CBA-12-1-T was also found in established mouse and hamster cell lines and in mouse organs. The low-molecular-weightmouse RNA has now been more carefully mapped and investigated for its origin. Surprisingly, we detected cellular RNAs when we extended the search to additional junction sites between adenovirus and cellular DNAs which we had previously cloned and sequenced (9). The scheme in Fig. 1 presents maps with the precise locations of several of these cellular RNAs at junction sites from hamster cell line HE5 (Fig. 1a) (14), from the mouse tumor CBA-12-1-T (Fig. 1b) (31), from the Ad12-induced hamster tumor line CLACl (Fig. 1c) (34), and from the Ad12-transformed hamster cell line HA12/7 (Fig. 1d) (40; Jessberger et al., in preparation). In the scheme, the preinsertion sites have been indicated, with the sites of viral DNA integration being marked by double-headed arrows. The integrated viral genomes (Ad2, Ad12) are also schematically indicated. In addition to cellular sequences, at which

viral DNAs had been integrated in transformed or tumor cells, transcriptional activity was also observed at a human cellular DNA sequence which had recombined with Ad12 DNA to give rise to a symmetric recombinant (SYREC2) DNA molecule (Fig. 1e) which was found to be packaged into virions (6). In this case, the human cellular RNA had a size of about 3 kb and was probably a product of RNA polymerase II, since this RNA could be translated into polypeptide chains in an in vitro translation system. We reasoned that the finding of transcriptional activity at all sites

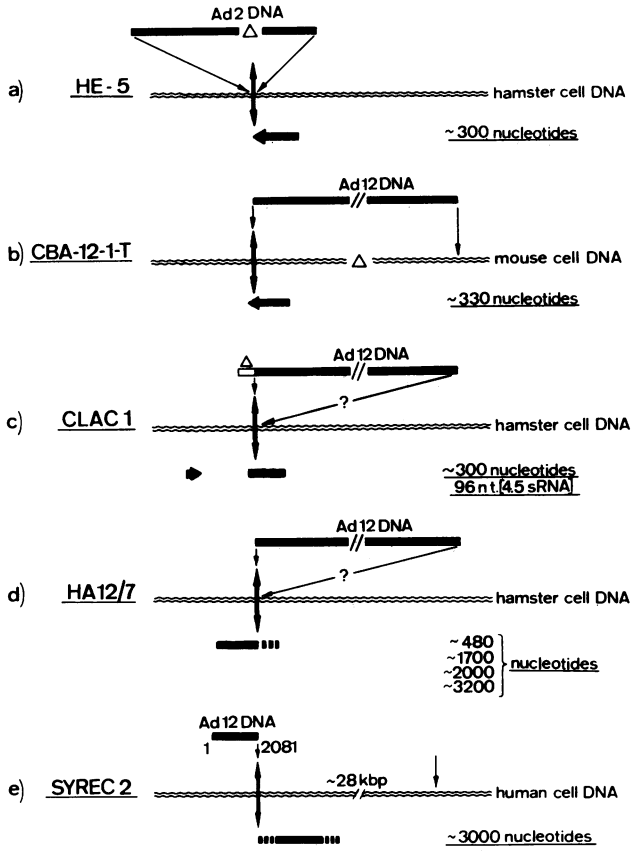

FIG. 1. Mapping of cellular transcripts with homologies to hamster, mouse, or human DNA at sites of integration or recombination with adenovirus DNA. The structures of integration sites of adenovirus DNA from the Ad2-transformed hamster cell line HE5 (a), from the Ad12-induced mouse tumor CBA-12-1-T (b), from the Ad12-induced hamster tumor line CLAC1 (c), and from the Ad12-transformed hamster cell line HA12/7 (d) and the structure of the site of recombination between Ad12 and human DNAs in the SYREC2 DNA molecule (e) are shown. The map positions of transcripts in cellular DNA are indicated by solid bars below individual maps. When determined, arrows indicate the polarity of transcripts. Symbols: △, deletions in viral or cellular DNA; —/ /—, interruptions in scale. The approximate lengths of the transcripts are given in nucleotides. In panel b, cellular DNA has been deleted at the site of insertion (31). In panel a, no cellular nucleotides were deleted (12). In panels c and d, it was not known whether cellular nucleotides were deleted. In panels a, b, and d, terminal deletions of viral nucleotides were <11 nucleotides or nonexistent and were therefore not indicated. In panel c, 174 nucleotides were deleted (△) at the left junction site. In panel e, the left-terminal 2081 nucleotides of Ad12 DNA were linked to human cellular DNA. The cellular transcript of about 3,000 nucleotides was not precisely mapped (indicated by broken bar).

of recombination-integration of adenovirus (foreign) DNA investigated in that respect was not coincidental but perhaps had significance for the mechanism of recombination. We therefore decided to analyze the transcription products in detail.

Transcription of mouse DNA at the insertion site from mouse tumor CBA-12-1-T. The preinsertion site corresponding to the CBA-12-1-T junction site had been cloned and partly sequenced previously (31). By using appropriate M13 (mp10 and mp11) subclones of the preinsertion site, we extended the sequence (Fig. 2a). Individual subclones which were sequenced are indicated schematically in Fig. 2b. A low-molecular-weight RNA with homology to this preinsertion site was found in mouse cells (14), and the RNA was mapped precisely on the preinsertion sequence. M13 subclones or isolated subfragments of this DNA sequence were used to localize the low-molecular-weight RNA by conventional RNA blot hybridization and by nuclease S1 protection analyses. A scheme indicating individual subclones and subfragments, as well as their locations and the results of S1 analyses of RNA blot hybridizations, is shown in Fig. 2c. The nucleotide sequence with homology to the small murine RNA comprised about 330 nucleotides and is marked in Fig. 2a. It was noted that a TATAAAA and a GGTCAAT signal preceded the sequence with homology to the small RNA (brackets in Fig. 2a). The ca. 330-nucleotide sequence spanned the insertion site of viral DNA (double-headed arrow in Fig. 2a and b) originally derived from the Ad12-induced mouse tumor CBA-12-1-T. By using procedures similar to the ones described previously (14), we estimated that in normal mouse cells, about 10 to 20 copies of the ca. 330-nucleotide RNA existed. This RNA was not poly(A)$^+$ and was fully sensitive to RNase (Fig. 3). The RNA was exclusively found in the cytoplasm. It was concluded that in mouse cells or mouse cell lines (mouse Ltk$^-$ cells) investigated, an RNA was isolated which was about 330 nucleotides in length and which exhibited homology to the cellular DNA at the site of Ad12 DNA insertion from the mouse tumor CBA-12-1-T. It was apparent from the scheme in Fig. 1b that the cellular sequence coding for the ca. 330-nucleotide RNA was deleted in the mouse tumor CBA-12-1-T. This deletion of cellular DNA comprised about 1.6 to 1.7 kb (31). Hence it was unlikely that this RNA was expressed in the mouse tumor. The deleted DNA sequence also showed homologies to small nuclear RNAs of about 120 nucleotides (data not shown).

Low-molecular-weight RNAs from the cellular sequence at the site of Ad12 DNA insertion in the Ad12-induced hamster tumor line CLAC1. The nucleotide sequences at the site of Ad12 DNA insertion in the Ad12-induced hamster tumor line CLAC1, as well as that from the preinsertion site from BHK21 hamster cells, were previously determined (34). The nucleotide sequence and a detailed restriction map of the preinsertion site are shown in Fig. 4a. Cytoplasmic RNA from hamster BHK21 cells or from mouse Ltk$^-$ cells was prepared and size fractionated on formaldehyde-agarose gels as described previously (30). Different fragments derived from the preinsertion site were used to investigate the location of possible transcripts from the hamster DNA preinsertion site (Fig. 4b and c). Depending on the probe used, two low-molecular-weight RNAs, one of 96 nucleotides and the other of about 300 nucleotides, were mapped (Fig. 4b and c). It could also be shown by procedures similar to those described in the legend to Fig. 3 that the 96- and the ca. 300-nucleotide RNAs were not poly(A)$^+$ (data not shown). In different experiments, the intensity of the ca.

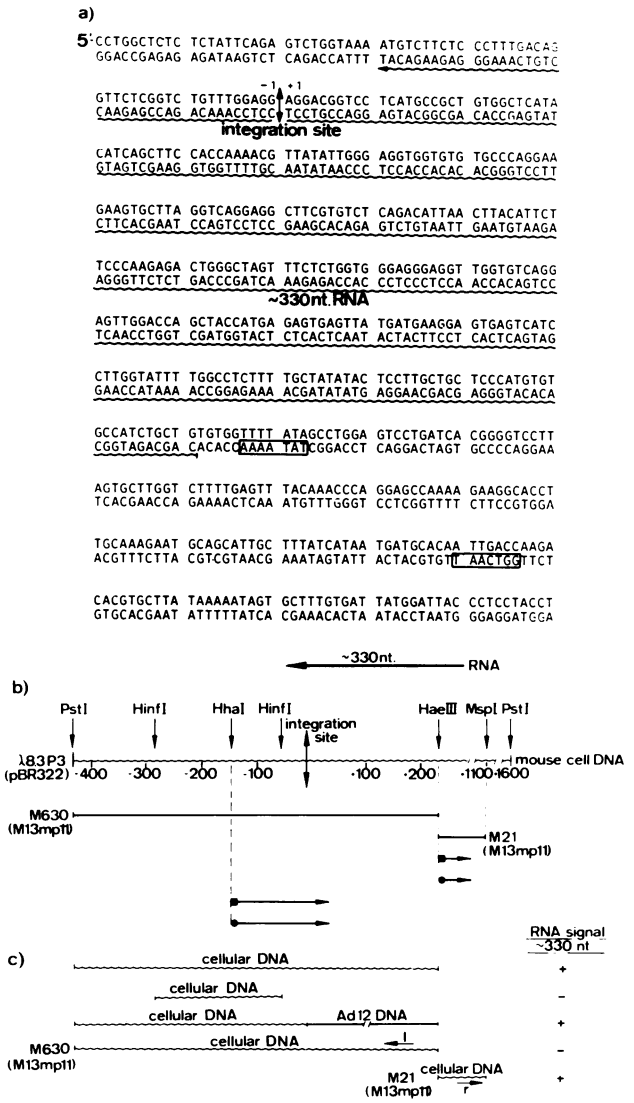

FIG. 2. Low-molecular-weight RNA with homology to mouse cell DNA corresponding to the site of Ad12 DNA insertion in the Ad12-induced mouse tumor CBA-12-1-T. Some of the details of the structure of this site are shown in Fig. 1b. As previously described (31), the preinsertion site from normal mouse cells, which corresponded to this insertion site, was cloned and sequenced. This sequence was now extended and is presented in panel a. The integration site (↕) and the position of homology to the ca. 330-nucleotide RNA are indicated in the sequence. The M13 subclones and isolated subclones used to determine this sequence, their map locations and the position of the ca. 330-nucleotide RNA are shown in panel b. The designated restriction endonuclease fragments were labeled at the 5′ (■) or the 3′ (●) terminus and were sequenced by the Maxam and Gilbert technique. The isolated subfragments which were used for nuclease S1 protection analysis or for RNA mapping by the RNA blot hybridization procedure are shown in panel c. The designations + and − refer to the presence or absence of a DNA signal in gel analyses when the indicated subclones or DNA fragments were used as ^{32}P-terminally labeled DNA fragments to protect total cytoplasmic RNA from mouse Ltk$^-$ cells by hybridization from S1 nuclease digestion or as hybridization probes. Experimental details have been described previously (4, 21).

300-nucleotide RNA varied to a certain degree. The 96-nucleotide RNA was also seen in the nuclear fraction.

It was apparent that in hamster cell DNA corresponding to the site of Ad12 DNA insertion in the Ad12-induced CLAC1 hamster tumor line, two low-molecular-weight cytoplasmic RNAs could be mapped. The larger, ca. 300-nucleotide, RNA spanned the insertion site, and the smaller, 96-nucleotide, RNA was located 481 nucleotides left of the insertion site (Fig. 4a). Computer comparisons of the nucleotide sequences in the regions of homology to known eucaryotic small RNA sequences revealed that at least 40 nucleotides in the 96-nucleotide RNA were identical to the 3′ terminus of a 4.5S hamster RNA (15, 16). Between nucleotides 1 and 40 in the sequence shown in Fig. 4a, there was one mismatch at position 34, owing to an additional C. From the orientation of this sequence, the direction of transcription of the 96-nucleotide RNA was derived. This sequence homology was first detected by I. Kruczek, S. Domrath, and S. Garbrecht (personal communication). The preinsertion sequence from hamster cells belonged to the repetitive sequences (34). It could therefore not be decided exactly from which sequence the RNAs described had originated.

RNAs with homologies to hamster cell DNA at the left junction site of Ad12 and hamster DNAs from the Ad12-transformed hamster cell line HA12/7. The patterns of viral DNA integration in cell line HA12/7 were described previously (35); the cloning and sequencing of the left junction sequence will be described here. The left-terminal junction fragment generated by *Pst*I cleavage was identified by hy-

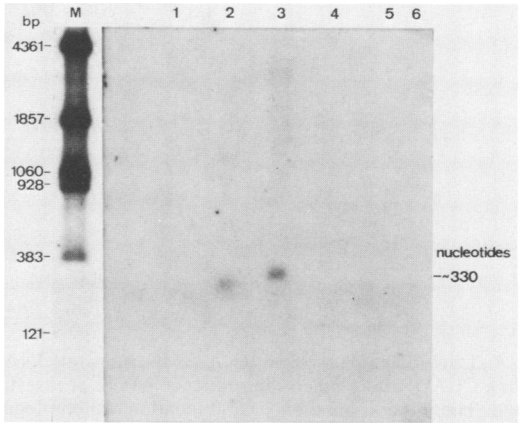

FIG. 3. Characteristics of low-molecular-weight mouse RNAs with homology to a cellular sequence corresponding to the site of insertion of Ad12 DNA in the tumor CBA-12-1-T. Cytoplasmic and nuclear RNAs were prepared from mouse Ltk$^-$ cells. Poly(A)$^+$ or poly(A)$^-$ RNA sequences were selected on polythymidylic acid coupled to Sepharose as described previously (2). Per gel slot, 50 μg of total or poly(A)$^-$-selected RNA or 2 μg of poly(A)$^+$-selected RNA was size fractionated on a 1.5% agarose gel containing 2.2 M formaldehyde (30). As a control, a portion of the nuclear or cytoplasmic RNA was treated with RNase (20 μg/ml, 37°C, 30 min). The RNAs were transferred to a nitrocellulose filter and hybridized (41) to the purified *Pst*I-*Hae*III subfragment (corresponding to M630) indicated in Fig. 2b as a probe which was ^{32}P labeled by nick translation (27). An autoradiogram of this RNA blot hybridization was shown. In lane M, a mixture of pBR322 DNA fragments generated by *Eco*RII or *Pst*I and NaOH denatured was coelectrophoresed as molecular weight marker. This portion of the filter was cut off and hybridized to ^{32}P-labeled pBR322 DNA. Lanes: 1, nuclear RNA; 2, total cytoplasmic RNA; 3, cytoplasmic RNA, poly(A)$^-$ selected; 4, cytoplasmic RNA, poly(A)$^+$ selected; 5 and 6, nuclear and cytoplasmic RNAs, respectively, treated with RNase.

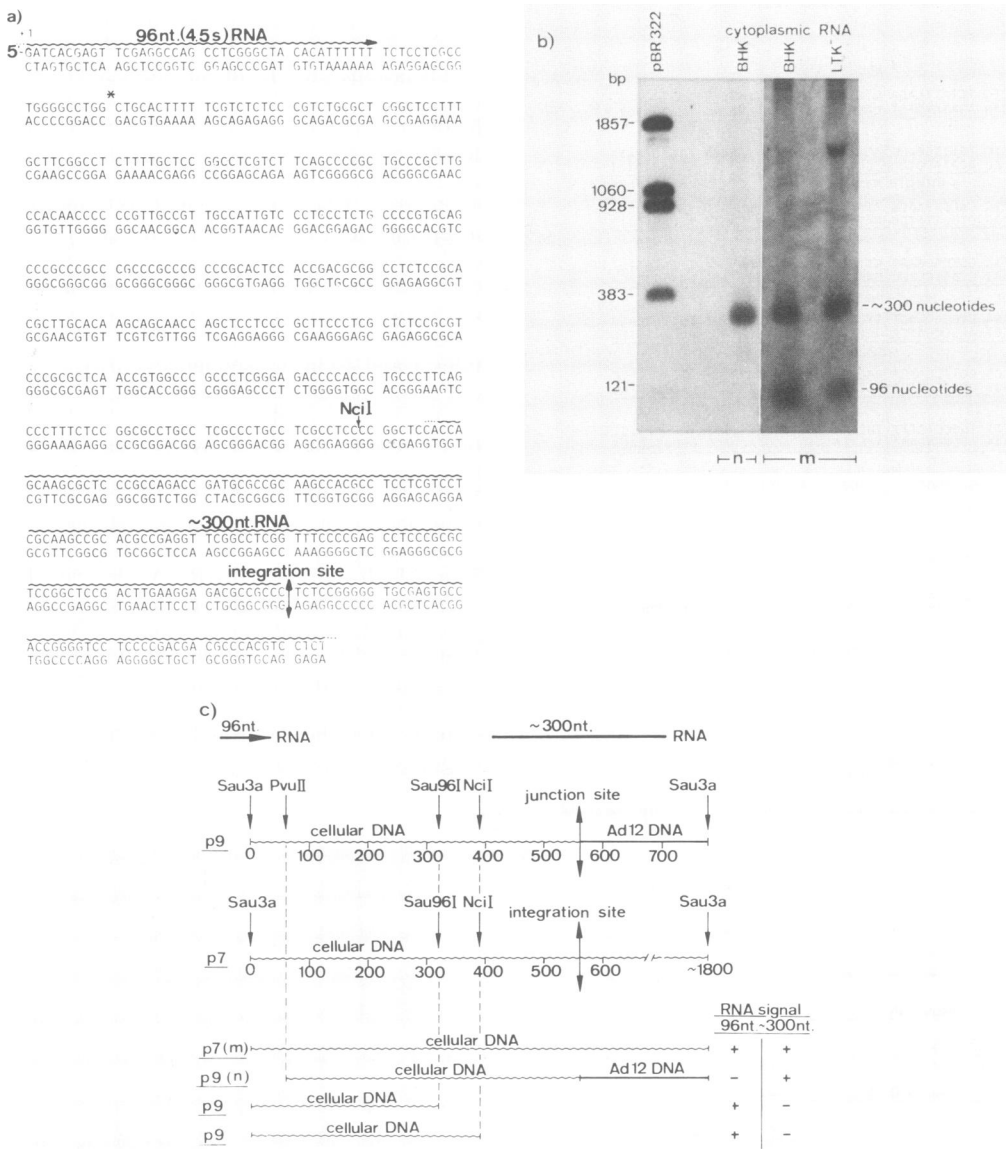

FIG. 4. Low-molecular-weight RNAs at the hamster DNA sequence corresponding to the Ad12 insertion site in the Ad12-induced hamster tumor line CLAC1. (a) Nucleotide sequence at site of insertion as determined previously (34). A detailed restriction map of this site is also presented. The integration site (↕) and location of homologies to the small RNAs are also indicated. The asterisk designates the position of a *Pvu*II site found previously (34) which was not apparent in this particular sequence. (b) Autoradiogram of an RNA blot hybridization experiment. pBR322 plasmid DNA was cut with *Eco*RII or *Pst*I, NaOH denatured, and coelectrophoresed as marker. Cytoplasmic RNAs from BHK21 cells or from mouse Ltk$^-$ cells were coelectrophoresed. Subsequently, RNAs on different parts of the filter were hybridized to fragment m or n as indicated in panel c. These DNA probes were excised from the vector, purified by gel electrophoresis, eluted, and ^{32}P labeled by nick translation. DNA fragments in the marker lane were hybridized to ^{32}P-labeled pBR322 DNA. Numbers at the left and right margins refer to RNA or fragment lengths in nucleotides. (c) Precise restriction map of hamster preinsertion site. The locations of the probes used in the experiment shown in panel b and the location of RNA homologies were also indicated. For the ca. 300-nucleotide RNA, the homology refers to the cellular DNA in the preinsertion site (p7) and not to Ad12 DNA. The scheme at the bottom describes fragment probes used to map the 96- and ca. 300-nucleotide RNAs more precisely. Depending on the probes used, RNA signals were found (+) or were absent (−).

bridizing the *Pst*I fragments of cellular DNA on a Southern blot to the left-terminal *Eco*RI C fragment (5.5 kilobase pairs [kbp]) of Ad12 DNA (Fig. 5a and b). The left-terminal *Pst*I off-size fragment of approximately 3.7 kbp (arrow in Fig. 5a) was ligated into the *Pst*I site of plasmid pBR322. Subsequently, this construct was cut with *Eco*RI and ligated to the *Eco*RI site-bounded termini (arms) of λgtWES DNA as previously described (7, 13). Upon in vitro packaging (18) of the DNA, recombinant λ plaques were isolated that hybridized to the left-terminal *Hin*dIII G fragment of Ad12 DNA and presumably included the left junction fragment of Ad12 DNA to hamster DNA (Jessberger et al., in preparation). By using a number of restriction endonucleases, we determined the physical map of the 3.7-kbp junction fragment (Fig. 5b). In these experiments, the immediate junction fragments were identified by hybridization to the *Hin*dIII G fragment of Ad12 DNA. The *Pst*I-*Bss*HII fragment spanning the site of linkage between Ad12 DNA and hamster DNA (Fig. 5b) was

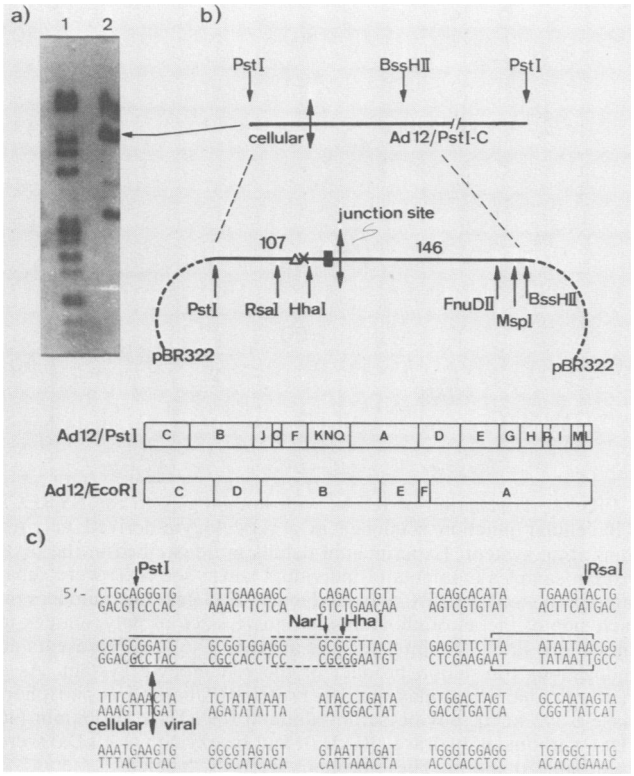

FIG. 5. Structure of the junction site between hamster cell DNA and the left end of Ad12 DNA in the Ad12-transformed cell line HA12/7. (a) Ad12 DNA (lane 1) or HA12/7 DNA (lane 2) was cut with *Pst*I, and the fragments were separated on a 1% agarose gel, transferred to a nitrocellulose filter (33), and hybridized (41) to ^{32}P-labeled (27) Ad12 DNA (lane 1) or the left-terminal *Eco*RI C fragment of Ad12 DNA (lane 2). (b) The off-size fragment comprising the left-junction fragment (arrow in lane 2 of panel a) was molecularly cloned as described in the text, and restriction maps were determined by standard procedures. The *Pst*I and *Eco*RI restriction maps of Ad12 DNA are also presented. Symbols: ■, ×, and △, TATATTAA, GGGCGCC, and CGGATGGCG sequences, respectively (see panel c. (c) Nucleotide sequence at the junction site between Ad12 and hamster cell DNAs. The site was designated by a double headed arrow. The sequences mentioned above were also indicated and show similarity to a TATA box (⌐¬), a possible SP1-binding site (- - -) (19), and an adenovirus DNA enhancer sequence (——) (17), respectively. There is no direct evidence that any of these sequences exert the suggested function in this context.

subcloned into pBR322 DNA, and the sequence of this insert (Fig. 5c) was determined by the dideoxy chain termination method (28) and with commercial pBR322 primers (42). In some experiments, the Maxam and Gilbert (22) method was also applied. The nucleotide sequence at this site differed from all other junction sequences between adenovirus and cellular DNAs hitherto determined in that not a single viral nucleotide had been deleted at the site of linkage. This is apparent from a comparison of the viral sequence at the site of junction (CTATCTATA. . .; Fig. 5c) with the authentic left-terminal Ad12 DNA sequence (39). As previously summarized, at all other adenoviral-cellular DNA linkage sites, viral nucleotides had been lost in the process of viral DNA integration (9). A more detailed report on the structure of Ad12 DNA molecules integrated into hamster cell DNA in cell line HA12/7 will be presented elsewhere (Jessberger et al., in preparation). For the studies reported here, only the structure of the left-terminal junction site (Fig. 5a to c) was relevant. The cellular DNA sequence at the immediate site of junction is probably of the unique type of DNA.

The 253-base-pair (bp) *Pst*I-*Bss*HII subfragment of the 3.7-kbp PstI junction fragment (Fig. 5b) contained 146 bp of Ad12 DNA, which is part of the inverted terminal repeat of Ad12 DNA and is not coding for any known function, and 107 bp of cellular DNA. This subfragment was ^{32}P labeled by nick translation and used as hybridization probe to search for homologous RNA sequences in total cytoplasmic or in poly(A)$^+$ or poly(A)$^-$-selected RNA from BHK21 or from HA12/7 hamster cells. RNA preparations from sources indicated in the legend to Fig. 6 were fractionated by electrophoresis on a 1% agarose gel containing 2.2 M formaldehyde. The RNA was then transferred to a nitrocellulose filter and hybridized to the subfragment probe mentioned above. The data revealed four hamster RNA size classes in HA12/7 cells, of approximately 3,200, 2,000, 1,700, and 480 nucleotides in length and with homologies to the hamster cell DNA fragment. The last size class was visible only upon prolonged exposure and is not visible in Fig. 6. Some of these RNAs might span the Ad12-cell DNA junction and might in part have homologies to Ad12 DNA. In BHK21 cells, only the 3,200-nucleotide RNA was apparent. We have not yet determined what caused the additional RNA size classes in HA12/7 cells; they might contain molecules comprising both cellular and viral sequences. The Ad12-hamster cell DNA

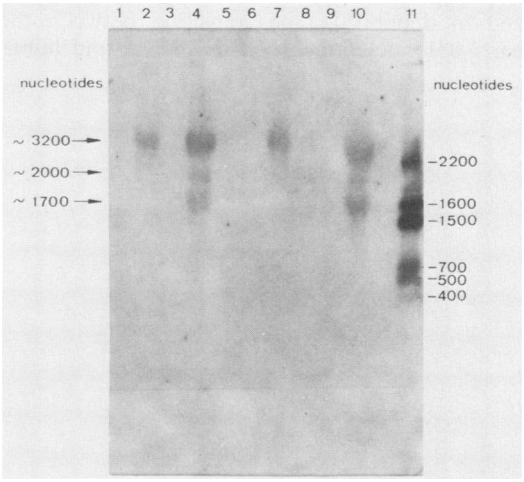

FIG. 6. Analyses of RNAs with homologies to the hamster cell DNA sequence at the site of linkage between the left terminus of Ad12 DNA and hamster cell DNA in cell line HA12/7. Some of the experimental details are described in the text. The autoradiogram presents the results of a blot hybridization experiment with the ^{32}P-labeled *Pst*I-*Bss*HII fragment in pBR322 characterized in Fig. 5b as probe and cytoplasmic RNA from the following sources: total cytoplasmic RNA from BHK21 cells treated with RNase (200 to 500 μg/ml, 25°C, 30 min) (lane 1); total cytoplasmic RNA from BHK21 cells (lane 2); total cytoplasmic RNA from HA12/7 cells treated with RNase (lane 3); total cytoplasmic RNA from HA12/7 cells (lane 4); poly(A)$^+$-selected RNA from BHK21 cells (lane 5); poly(A)$^-$-selected RNA from BHK21 cells treated with RNase (lane 6); poly(A)$^-$-selected RNA from BHK21 cells (lane 7); poly(A)$^+$-selected RNA from HA12/7 cells (lane 8); poly(A)$^-$-selected RNA from HA12/7 cells treated with RNase (lane 9); poly(A)$^-$-selected RNA from HA12/7 cells (lane 10); and DNA marker fragments (lane 11). pBR322 plasmid DNA was cut with either *Hin*fI or *Rsa*I, and the fragments were mixed, NaOH denatured, and coelectrophoresed. The numbers at either side represent RNA and DNA fragment sizes in nucleotides.

*Pst*I-*Bss*HII junction fragment of 253 bp (Fig. 5b) was subcloned into M13 DNAs mp18 or mp19, and these DNAs were hybridized to cytoplasmic RNA from HA12/7 cells.

Nuclease S1 protection preserved DNA fragments of that polarity, which indicated that at least some of the transcripts started within the 146 terminal Ad12 nucleotides and extended outward into cellular DNA. These results demonstrated that at least some of the transcripts described here were derived from cellular DNA sequences. The hybridization data (Fig. 6) also attested to the RNase sensitivity of these RNA size classes and demonstrated that these RNAs were not poly(A)$^+$. It is concluded that several size classes of cytoplasmic hamster cell RNA exhibit homologies at least to the 107 nucleotide pairs of cellular DNA immediately abutting the left terminus of integrated Ad12 DNA (Fig. 5c). The RNAs are poly(A)$^-$, except for the 480-nucleotide RNA, which is poly(A)$^+$. A signal of about 3,200 nucleotides in length was also detected in nuclear RNA preparations from BHK21 and HA12/7 hamster cells.

Transcriptional activity of a human KB cell DNA sequence derived from the site of linkage between Ad12 DNA and human cell DNA in the symmetric recombinant SYREC2. From human KB cells productively infected with Ad12, symmetric recombinants between the left terminus of Ad12 DNA and human cell DNA were isolated and characterized (5, 6). The recombinant DNA had been generated naturally in infected cells, had approximately the length of Ad12 DNA, and was encapsidated into virionlike particles. The DNA from one of these recombinants, SYREC2, had been analyzed in detail (5). The transition between the left-terminal 2,081 nucleotides of Ad12 DNA and human cell DNA was cloned as a 1.4-kbp fragment (termed the SYREC2-1.4-kbp fragment), and a sequence of 329 nucleotides encompassing the site of linkage was determined (5). The question arose whether this particular cellular DNA sequence of unknown location and function in the human genome was transcribed in human cells. It had been shown that the human cell DNA sequence at the site of junction to Ad12 DNA (SYREC2-1.4-kbp fragment) was represented only a few times per human cell and hence was probably of the low-abundance type (5). Total cytoplasmic RNA from human KB cells (Fig. 7, lane 2), as well as poly(A)$^-$- or poly(A)$^+$-selected RNA (lanes 3 and 4, respectively), was fractionated on agarose gels containing 2.2 M formaldehyde and transferred to a nitrocellulose filter. The RNA on this filter was then probed with the ^{32}P-labeled SYREC2-1.4-kbp fragment cloned in pBR322 DNA. The results (Fig. 7) demonstrated that an RNA of about 3 kb had homology to the human cellular junction sequence. It was also apparent that the bulk of the ca. 3-kb RNA was poly(A)$^+$. The fraction of poly(A)$^-$ RNA yielded no signal of homology at the 3-kb size stratum upon hybridization (Fig. 7). In some experiments, a low-molecular-weight RNA of about 400 nucleotides in length could also be seen in cytoplasmic RNA. This low-molecular-weight RNA seemed not to be poly(A)$^+$. Moreover, both signals disappeared completely when the RNA was treated with RNase prior to being fractionated on agarose gels (Fig. 7, lane 1). Results similar to those shown in Fig. 7 were also obtained with cytoplasmic RNA from human HeLa cells.

The cytoplasmic location, the content of poly(A) residues, and the derivation from a cellular sequence of low abundance suggested that the human ca. 3-kb RNA might be the transcription product of a cellular gene. Hence, the cellular DNA sequence linked to Ad12 DNA in the SYREC2 recombinant was possibly part of this presumptive gene. Extensive

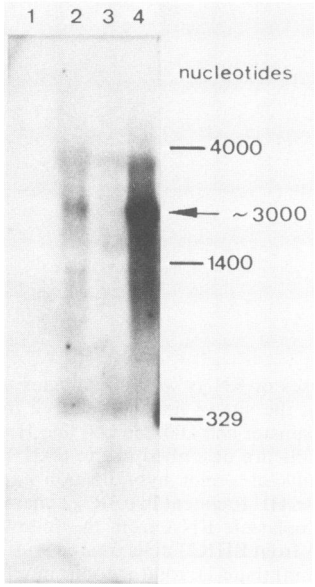

FIG. 7. Cytoplasmic RNA from human KB cells with homology to the cellular sequence at the junction with Ad12 DNA from the SYREC2 molecule. Cytoplasmic RNA was prepared from human KB cells and analyzed as described in the text. RNA samples electrophoresed on a 1% agarose–2.2 M formaldehyde gel were the following: total cytoplasmic RNA treated with RNase (10 mg/ml) (lane 1); total cytoplasmic RNA (lane 2); poly(A)$^-$-selected RNA (lane 3); and poly(A)$^+$-selected RNA (lane 4). The SYREC2-1.4-kbp fragment in pBR322 DNA was used as the ^{32}P-labeled hybridization probe. An appropriate size marker was used (not shown). The numbers at the right margin refer to RNA and DNA fragment lengths in nucleotides.

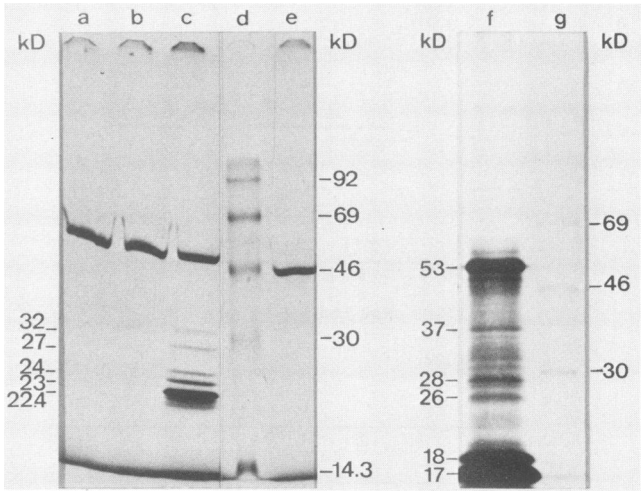

FIG. 8. Translation of RNAs with homologies to the SYREC2 KB cellular junction sequence in a reticulocyte-derived in vitro translation system. Experimental details are described in the text. Protein samples (contents of individual lanes; see text) were separated on a standard 15% polyacrylamide-SDS gel. The numbers on each side of the autoradiograms designate sizes of polypeptides in kilodaltons (kD). The intense band at around 46 kDa represents an endogenous translation product of the reticulocyte system. As a mixture of molecular mass marker proteins (Amersham), phosphorylase *b* (92 kDa), bovine serum albumin (69 kDa), ovalbumin (46 kDa), carbonic anhydrase (30 kDa), and lysozyme (14.3 kDa) were used (lanes d and g). These proteins were ^{14}C labeled.

TABLE 1. Cellular RNAs at viral integration sites

Origin of junction site	Copy number/cell		Size (nucleotides)[b]	Nucleus	Cytoplasm	
	Gene	Transcript			poly(A)$^-$	poly(A)$^+$
HE5	Single copy	20–30	ca. 300	−	−	+
			(ca. 150/ca. 80)	−	+	−
CBA12-1-T	Single copy	10–20	331	−	+	−
			(ca. 120)	+	ND[a]	ND
CLAC1	Middle repetitive	10^5	96	+	+	−
		20	ca. 300	−	+	−
HA12/7	Single copy	<20	ca. 480, ca. 1,700, ca. 2,000	−	+	− (+)
			ca. 3,200	+	+	−
SYREC2	Middle repetitive	ND	ca. 3,000	−	− (+)	+

[a] ND, Not determined.
[b] Size classes in parentheses designate minor populations.

computer searches of the junction sequence of 304 bp of cellular DNA (5) did not reveal any identity to known mammalian genes.

The coding capacity of the human RNA with homology to the SYREC2-1.4-kbp fragment was further tested by in vitro translation experiments with a reticulocyte-derived system. Cytoplasmic KB cell RNA was hybridized to SYREC2-1.4-kbp DNA which had been fixed to nitrocellulose filters. The selected RNA sequences were subsequently translated in a cell-free system from rabbit reticulocytes by using standard procedures and [^{35}S]methionine to tag the synthesized polypeptides (see Materials and Methods). The optimal temperature of hybridization selection (11) for the RNA homologous to the SYREC2-1.4-kbp fragment was experimentally determined and proved to be 60°C (Fig. 8, lane c). Hybridization selection at 50°C (lane a) or 55°C (lane b) yielded no translatable RNA. By using ^{14}C-labeled commercial marker proteins (Amersham Corp.) as size markers (lane d), which were coelectrophoresed on the same polyacrylamide gel, we estimated the molecular mass of the major translation product to be 22.4 kilodaltons (kDa). Minor translation products exhibited molecular masses of 32, 27, 24, and 23 kDa. Incubation of the reticulocyte extract without added RNA yielded the known endogenous protein band but no specific products (lane e) and thus provided a negative control experiment. In a positive control (lane f), RNA was in vitro translated that had been isolated from the cytoplasm of human KB cells at 20 h after the infection with Ad12 and was hybrid selected on the *Eco*RI C fragment which comprised the left terminus of the Ad12 genome. The results demonstrated the 37-, 28.5-, and 26-kDa E1A proteins and the 53-, 18-, and 17-kDa E1B proteins of Ad12 DNA relative to the sizes of marker polypeptides (lane g).

The finding that a 3,000-nucleotide RNA yielded a major translation product of only 22.4 kDa indicated that portions of the mRNA apparently constituted nontranslated sequences. It is worth commenting upon the absence of translatable RNA when hybridization temperatures of 50 or 55°C were used. The reason for this narrow selection is not understood. The results of analyses on human cytoplasmic RNAs from KB or HeLa cells with homologies to the cellular DNA in the SYREC2-1.4-kbp fragment provide evidence for a coding function of this RNA and suggest that the cellular DNA sequence in the symmetric recombinant was derived from a functional human gene which is actively transcribed and poly(A)$^+$ in uninfected human cells. Further work will be directed toward the isolation and characterization of this cellular DNA sequence.

DISCUSSION

Little is known about the mechanisms and the general significance of recombination in mammalian cells. We have performed structural analyses on sites of recombination between mammalian cell DNA (hamster, mouse, and human) and foreign DNA which had been inserted into the mammalian genomes. As a foreign DNA model, adenovirus DNA has been investigated. Comparisons of nucleotide sequences at a number of sites for recombination between adenovirus and cellular DNA have not provided evidence that adenovirus DNA insertion occurs at unique, highly specific cellular DNA sequences (9, 29). Computer comparisons of nucleotide sequences at the sites of adenovirus DNA insertion from several transformed or tumor cell lines have not shown identical DNA sequences other than patches of up to dodecanucleotides (9). These patch homologies at the sites of recombination as prerequisites of homologous recombination have not always been observed. It has previously been postulated that patch homologies might exert a stabilizing effect on recombination complexes (7, 13, 31, 34, 36). Although definite statements about the type of recombination in foreign DNA insertion would appear to be premature, there are examples consistent with both the homologous and heterologous mechanisms of recombination.

On the basis of analyses of transcriptional activities of cellular DNA sequences involved in recombination with foreign (adenoviral) DNA, it appears plausible that a transcriptionally activated state of cellular DNA sequences is a necessary but not sufficient precondition for recombination events at these cellular sites. The 96-nucleotide RNA with homology to the CLAC1 preinsertion site from BHK21 cells is expressed at a level of 10^5 copies per cell.

We also conducted a computer search on all the cellular DNA sequences, which the described transcripts showed homologies to, for identities or similarities to known eucaryotic sequences. These computer-aided comparisons did not reveal any known sequences, except for the 4.5S RNA from hamster cells (15, 16) already mentioned. The 96-nucleotide

RNA from hamster cells with homology to the cellular DNA close to the junction site in the Ad12-induced hamster tumor line CLAC1 was identical in sequence to part of the 4.5S RNA. The biological function of this RNA is not known.

The most important characteristics of each of the different transcripts analyzed in this report are summarized in Table 1. The number of RNA copies found per cell varied considerably depending on the cellular DNA sequence that corresponded to the sites of insertion in different cell lines. It was apparent from the data presented in Table 1 that the copy number of RNA molecules detectable per cell correlated in many instances to the extent of abundance of the homologous DNA sequence. It was uncertain whether a simple quantitative relationship could account for the differences in transcriptional activities at individual cellular sites. More complicated regulatory mechanisms are also conceivable.

It may be important to mention that the adenovirus-transformed cell lines, from which the sites of viral DNA insertion have been isolated, are of course highly selected products of virus-cell DNA recombination events. We do not intend to extrapolate from these investigations and do not mean to extend the conclusions to all recombination events in mammalian cells. However, these mechanisms, although different in some aspects, may have certain features in common.

ACKNOWLEDGMENTS

We are indebted to Jochen Schellner for performing computer analyses of nucleotide sequences and to Hanna Mansi-Wothke for preparing cell culture media and maintaining cells in culture. Petra Böhm performed expert editorial work.

This research was supported by the Federal Ministry of Research and Technology, Bonn-Bad Godesberg, Federal Republic of Germany (grant BCT 0365-2) and by the Deutsche Forschungsgemeinschaft through grant SFB74-C1.

LITERATURE CITED

1. **Alwine, J. C., D. J. Kemp, and G. R. Stark.** 1977. Method for detection of specific RNAs in agarose gels by transfer to diazobenzyloxymethyl-paper and hybridization with DNA probes. Proc. Natl. Acad. Sci. USA **74**:5350–5354.
2. **Aviv, H., and P. Leder.** 1972. Purification of biologically active globin messenger RNA by chromatography on oligothymidylic acid-cellulose. Proc. Natl. Acad. Sci. USA **69**:1408–1412.
3. **Bablanian, R., H. J. Eggers, and I. Tamm.** 1965. Studies on the mechanism of poliovirus-induced cell damage. I. The relation between poliovirus-induced metabolic and morphological alterations in cultured cells. Virology **26**:100–113.
4. **Berk, A. J., and P. A. Sharp.** 1977. Sizing and mapping of early adenovirus mRNAs by gel electrophoresis of S1 endonuclease-digested hybrids. Cell **12**:721–732.
5. **Deuring, R., and W. Doerfler.** 1983. Proof of recombination between viral and cellular genomes in human KB cells productively infected by adenovirus type 12: structure of the junction site in a symmetric recombinant (SYREC). Gene **26**:283–289.
6. **Deuring R., G. Klotz, and W. Doerfler.** 1981. An unusual symmetric recombinant between adenovirus type 12 DNA and human cell DNA. Proc. Natl. Acad. Sci. USA **78**:3142–3146.
7. **Deuring R., U. Winterhoff, F. Tamanoi, S. Stabel, and W. Doerfler.** 1981. Site of linkage between adenovirus type 12 and cell DNAs in hamster tumour line CLAC3. Nature (London) **293**:81–84.
8. **Doerfler, W.** 1969. Nonproductive infection of baby hamster kidney cells (BHK21) with adenovirus type 12. Virology **38**:587–606.
9. **Doerfler, W., R. Gahlmann, S. Stabel, R. Deuring, U. Lichtenberg, M. Schulz, D. Eick, and R. Leisten.** 1983. On the mechanism of recombination between adenoviral and cellular DNAs: The structure of junction sites. Curr. Top. Microbiol. Immunol. **109**:193–228.
10. **Dretzen, G., M. Bellard, P. Sassone-Corsi, and P. Chambon.** 1981. A reliable method for the recovery of DNA fragments from agarose and acrylamide gels. Anal. Biochem. **112**:295–298.
11. **Esche, H., H. Lübbert, B. Siegmann, and W. Doerfler.** 1982. The translational map of the *Autographa californica* nuclear polyhedrosis virus (AcNPV) genome. EMBO J. **1**:1629–1633.
12. **Gahlmann, R., and W. Doerfler.** 1983. Integration of viral DNA into the genome of the adenovirus type 2-transformed hamster cell line HE5 without loss or alteration of cellular nucleotides. Nucleic Acids Res. **11**:7347–7361.
13. **Gahlmann, R., R. Leisten, L. Vardimon, and W. Doerfler.** 1982. Patch homologies and the integration of adenovirus DNA in mammalian cells. EMBO J. **1**:1101–1104.
14. **Gahlmann, R., M. Schulz, and W. Doerfler.** 1984. Low molecular weight RNAs with homologies to cellular DNA at sites of adenovirus DNA insertion in hamster or mouse cells. EMBO J. **3**:3263–3269.
15. **Harada, F., and N. Kato.** 1980. Nucleotide sequences of 4.5S RNAs associated with poly(A)-containing RNAs of mouse and hamster cells. Nucleic Acids Res. **8**:1273–1285.
16. **Harada, F., Y. Takeuchi, and N. Kato.** 1986. Molecular cloning and in vitro transcription of rat 4.5S RNA genes. Nucleic Acids Res. **14**:1629–1642.
17. **Hearing, P., and T. Shenk.** 1983. The adenovirus type 5 E1A transcriptional control region contains a duplicated enhancer element. Cell **33**:695–703.
18. **Hohn, B.** 1979. In vitro packaging of λ and cosmid DNA. Methods Enzymol. **68**:299–309.
19. **Jones, K. A., and R. Tjian.** 1985. Sp1 binds to promoter sequences and activates herpes simplex virus 'immediate-early' gene transcription in vitro. Nature (London) **317**:179–182.
20. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
21. **Lübbert, H., and W. Doerfler.** 1984. Transcription of overlapping sets of RNAs from the genome of *Autographa californica* nuclear polyhedrosis virus: a novel method for mapping RNAs. J. Virol. **52**:255–265.
22. **Maxam, A. M., and W. Gilbert.** 1977. A new method for sequencing DNA. Proc. Natl. Acad. Sci. USA **74**:560–564.
23. **Messing, J., and J. Vieira.** 1982. A new pair of M13 vectors for selecting either DNA strand of double-digest restriction fragments. Gene **19**:269–276.
24. **Morrison, D. A.** 1979. Transformation and preservation of competent bacterial cells by freezing. Methods Enzymol. **68**:326–331.
25. **Pelham, H. R. B., and R. J. Jackson.** 1976. An efficient mRNA-dependent translation system from reticulocyte lysates. Eur. J. Biochem. **67**:247–256.
26. **Ricciardi, R. P., J. S. Miller, and B. E. Roberts.** 1979. Purification and mapping of specific mRNAs by hybridization-selection and cell-free translation. Proc. Natl. Acad. Sci. USA **76**: 4927–4931.
27. **Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labeling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I. J. Mol. Biol. **113**:237–251.
28. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
29. **Schellner, J., K. Stüber, and W. Doerfler.** 1986. Computer analyses on the structure of junction sites between adenovirus DNA and cellular DNA. Biochim. Biophys. Acta **867**:114–123.
30. **Schirm, S., and W. Doerfler.** 1981. Expression of viral DNA in adenovirus type 12-transformed cells, in tumor cells, and in revertants. J. Virol. **39**:694–702.
31. **Schulz, M., and W. Doerfler.** 1984. Deletion of cellular DNA at site of viral DNA insertion in the adenovirus type 12-induced mouse tumor CBA-12-1-T. Nucleic Acids Res. **12**:4959–4976.
32. **Smithies, O., R. G. Gregg, S. S. Boggs, M. A. Koralewski, and R. S. Kucherlapati.** 1985. Insertion of DNA sequences into the

human chromosomal β-globin locus by homologous recombination. Nature (London) **317**:230–234.
33. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
34. **Stabel, S., and W. Doerfler.** 1982. Nucleotide sequence at the site of junction between adenovirus type 12 DNA and repetitive hamster cell DNA in transformed cell line CLAC1. Nucleic Acids Res. **10**:8007–8023.
35. **Stabel, S., W. Doerfler, and R. R. Friis.** 1980. Integration sites of adenovirus type 12 DNA in transformed hamster cells and hamster tumor cells. J. Virol. **36**:22–40.
36. **Stringer, J. R.** 1981. Integrated simian virus 40 DNA: nucleotide sequences at cell-virus recombinant junctions. J. Virol. **38**:671–679.
37. **Strohl, W. A., H. Rouse, K. Teets, and R. W. Schlesinger.** 1970. The response of BHK21 cells to infection with type 12 adenovirus. III. Transformation and restricted replication of superinfecting type 2 adenovirus. Arch. Gesamte Virusforsch. **31**:93–112.
38. **Subramani, S., and P. Berg.** 1983. Homologous and nonhomologous recombination in monkey cells. Mol. Cell. Biol. **3**:1040–1052.
39. **Sugisaki, H., K. Sugimoto, M. Takanami, K. Shiroki, I. Saito, H. Shimojo, Y. Sawada, Y. Uemizu, S. Uesugi, and K. Fujinaga.** 1980. Structure and gene organization in the transforming HindIII-G fragment of Ad12. Cell **20**:777–786.
40. **Sutter, D., M. Westphal, and W. Doerfler.** 1978. Patterns of integration of viral DNA sequences in the genomes of adenovirus type 12-transformed hamster cells. Cell **14**:569–585.
41. **Wahl, G. M., M. Stern, and G. R. Stark.** 1979. Efficient transfer of large DNA fragments from agarose gels to diazobenzyloxymethyl-paper and rapid hybridization by using dextran sulfate. Proc. Natl. Acad. Sci. USA **76**:3683–3687.
42. **Wallace, B. R., M. J. Johnson, S. V. Suggs, K. Miyoshi, R. Bhatt, and K. Itakura.** 1981. A set of synthetic oligodeoxyribonucleotide primers for DNA sequencing in the plasmid vector pBR322. Gene **16**:21–26.
43. **Wilson, J. H., P. B. Berget, and J. M. Pipas.** 1982. Somatic cells efficiently join unrelated DNA segments end-to-end. Mol. Cell. Biol. **2**:1258–1269.
44. **Winocour, E., and I. Keshet.** 1980. Indiscriminate recombination in simian virus 40-infected monkey cells. Proc. Natl. Acad. Sci. USA **77**:4861–4865.