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Low molecular weight RNAs with homologies to cellular DNA at sites of adenovirus DNA insertion in hamster or mouse cells

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The adenovirus type 2 (Ad2)-transformed hamster cell line HE5 contains one or very few integrated copies of Ad2 DNA. At the site of insertion of Ad2 DNA, the cellular DNA sequence has been completely preserved and has homologies to small unpolyadenylated, cytoplasmic RNAs of 300 nucleotides in length and to minority populations of smaller RNAs present in HE5 cells and in normal hamster cells. The 300-nucleotide RNA is present on average in ~20 copies per cell. This RNA, and shorter RNAs, reveal homologies to the hamster DNA sequence of ~400 nucleotides to the right of the site of insertion of Ad2 DNA, which is present in one or very few copies per genome. The nucleotide sequence of the DNA segment homologous to this RNA does not contain open reading frames in excess of a sequence encoding 18 amino acids. Thus, it is unlikely that the small RNAs are actually translated and their function is unknown. The nucleotide sequence does not exhibit similarities to known low mol. wt. RNAs of eukaryotic origin. The low mol. wt. cellular RNA has been found in HE5 cells, in other hamster cell lines and organs, and also in mouse cells. There are differences with respect to size and abundance in the RNAs smaller than 300 nucleotides between HE5 cells and LSH hamster embryo cells. The adenovirus type 12 (Ad12)-induced mouse tumor CBA-12-1-T carries >30 copies of integrated Ad12 DNA. The cellular DNA sequence at the site of Ad12 DNA insertion exhibits homologies to small RNAs (~300 nucleotides long) from mouse cells. The finding of low mol. wt. RNAs with homologies to cellular DNA at the site of insertion of adenovirus DNA suggests that foreign DNA can perhaps be more easily inserted at cellular sites which are actively transcribed.

Key words: integration site/low molecular weight RNAs/mechanism of integration/Ad2-transformed hamster cells/Ad12-induced mouse tumor

Introduction

The adenovirus type 2 (Ad2)-transformed hamster cell line HE5 originated from transformation of primary LSH hamster embryo cells with u.v.-inactivated Ad2 (Johansson *et al.*, 1978; Cook and Lewis, 1979). The pattern of Ad2 DNA integration has been described in detail (Johansson *et al.*, 1978; Vardimon and Doerfler, 1981; Gahlmann and Doerfler, 1983), and the viral-specific RNAs expressed in HE5 cells have been analyzed by *in vitro* translation (Esche, 1982). HE5 cells carry one or very few copies of Ad2 DNA with a major internal deletion encompassing map units 35 – 82 (Gahlmann *et al.*, 1982; Gahlmann and Doerfler, 1983). Both the right and left viral-cellular junctions, as well as the internal link between the two fragments of Ad2 DNA, have been cloned and sequenced (Gahlmann and Doerfler, 1983). Similarly, the unoccupied site corresponding to the site of viral DNA insertion has been cloned and sequenced (Gahlmann and Doerfler, 1983). This cellular sequence is present in one or very few copies per cell (Gahlmann *et al.*, 1982). Figure 1 summarizes the arrangement of the internally deleted Ad2 genome in unique hamster cell DNA. The Ad2 DNA has been inserted into a unique cellular DNA sequence without the loss, rearrangement or amplification of a single cellular nucleotide. Cellular nucleotides at the site of insertion are not duplicated, indicating that Ad2 DNA insertion is different from that of retroviral DNA (Majors and Varmus, 1981). At the right site of the junction eight nucleotides, and at the left site 10 nucleotides of viral DNA have been deleted. At the internal junction linking the right and left truncated fragments of Ad2 DNA, a $\frac{GT}{CA}$ dinucleotide of unknown origin has been inserted. A model has been proposed in which the terminal viral protein, or the terminal viral DNA sequences, or both, play an important role in the recombination of viral DNA with cellular DNA (Gahlmann and Doerfler, 1983).

The comparison of cellular DNA sequences at several different sites of adenovirus DNA insertion in hamster, mouse and human DNAs failed to provide any evidence for a common nucleotide sequence at the sites of recombination (Doerfler *et al.*, 1983). The insertion mechanism appears to be governed by parameters other than sequence identities. Patch homologies may help to stabilize the recombination complex in some instances (Stabel and Doerfler, 1982; Doerfler *et al.*, 1983; Schulz and Doerfler, 1984). The recombination mechanism may also be capable of operating on the basis of heterologous recombination. Perhaps one of the decisive features of a cellular site becoming involved in recombination is its chromatin structure and whether or not it is opened up for contacts with foreign DNA.

Here we describe low mol. wt. RNAs which have homologies to the hamster cell sequence immediately to the right of the site of Ad2 DNA insertion in cell line HE5. This RNA is predominantly cytoplasmic, not polyadenylated and is widely distributed in normal tissues from hamster. RNAs with homologies to that sequence have also been found in mouse cells, but not in human, insect or *Xenopus laevis* cells. The RNA sequence lacks significant open reading frames. The cellular DNA sequence at the site of Ad12 DNA insertion in the Ad12-induced mouse tumor CBA-12-1-T is also homologous to low mol. wt. RNAs in mouse cells. It is conceivable that the insertion mechanism for adenovirus (foreign) DNA selects areas of transcriptional activity of the cell.

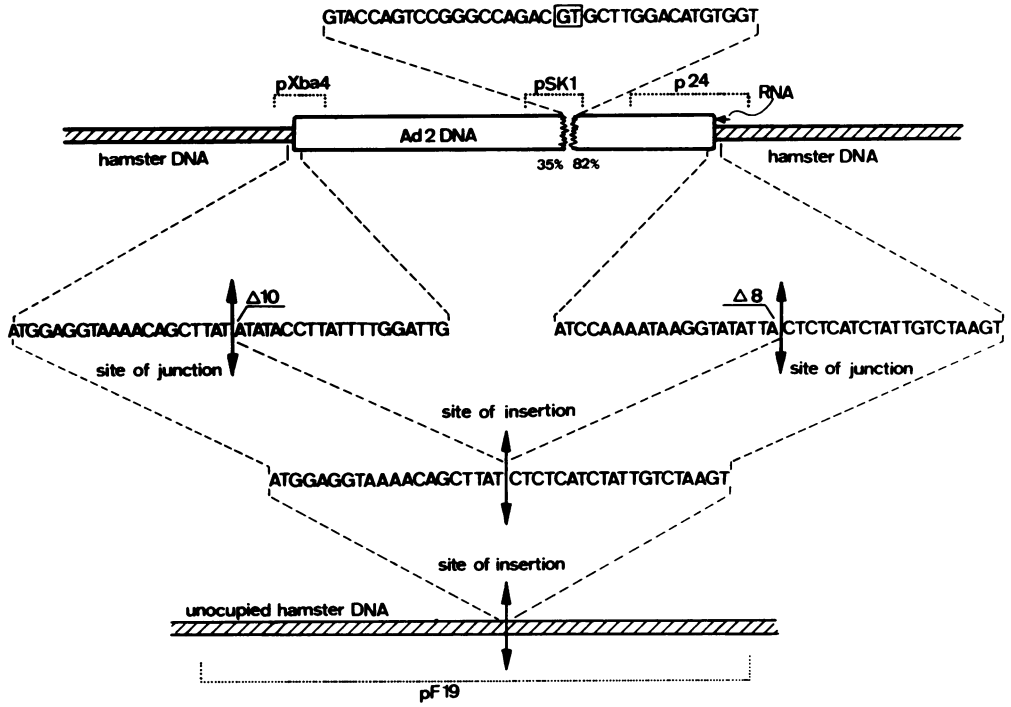

Fig. 1. Integrated Ad2 genome in unique hamster cell DNA in cell line HE5. The horizontal arrow designates size, location, and direction of transcription of the low mol. wt. cellular RNA described in this report. The sequence data summarized here have been published elsewhere (Gahlmann *et al.*, 1982; Gahlmann and Doerfler, 1983). The map positions of the major clones used in localizing the low mol. wt. cellular RNA investigated in this study have also been indicated (pF19, pXba4, p24). Clone pSKl spans the internal viral deletion.

Results

Transcripts homologous to cellular DNA at the site of junction

The cloned unique cellular DNA sequences surrounding the sites of junction (Gahlmann and Doerfler, 1983) were used to probe RNAs from the Ad2-transformed hamster line HE5, from additional hamster and mouse cell lines, and from hamster organs and embryonic hamster tissue for the presence of homologous transcripts. Total intracellular RNA was extracted from HE5 cells and from 14-day old hamster embryos. Amounts of 50 μg of RNA were fractionated by electrophoresis on 1.5% agarose gels containing 2.2 M formaldehyde (Schirm and Doerfler, 1981) and transferred to nitrocellulose filters. Equivalent portions of total RNA were treated with RNase prior to electrophoresis to establish the absence of DNA contaminants. DNA restriction fragments of plasmid pUR2 were used as size markers in all experiments. The plasmid clone pF19 (cf. Figure 1), which was derived from the unoccupied cellular site, was ^{32}P-labeled by nick translation and was used to detect RNA transcripts from that region. The data (Figure 2a) demonstrate RNA populations of ~300, 150, and perhaps 80 nucleotides which are sensitive to RNase treatment. An estimate outlined in the legend to Figure 2 suggests that, on average, each HE5 cell contains ~20 copies of this low mol. wt. RNA.

The total intracellular RNA was fractionated into poly(A)$^+$ and poly(A)$^-$ populations, and both fractions were analyzed for the presence of RNA with homologies to the cellular DNA at the junction site. The results (Figure 2b) show that in HE5 cells a low mol. wt. 300-nucleotide RNA is transcribed with homologies to the cellular part of the junction site, and the majority of these RNA molecules is poly(A)$^-$.

Low mol. wt. cellular RNAs in size classes of ~300, 160, 140 and 80 nucleotides and with homologies to the cellular DNA at the right site of junction (Figure 1) were also found in LSH hamster embryo cells. There were minor but distinct differences in sizes and relative amounts of the small RNAs between HE5 and primary hamster cells. In some experiments, the smaller size classes of RNAs were difficult to detect. However, the smaller size RNAs appeared to predominate in LSH hamster embryo cells as compared with HE5 cells. It cannot be excluded that the smaller RNAs represent (specific) degradation products of the 300-nucleotide RNA.

Precise mapping and polarity of the low mol. wt. cellular RNA

Different parts of the sites of junction between Ad2 DNA and hamster cell DNA from cell line HE5 were cloned, and smaller subclones were established (Gahlmann *et al.*, 1982; Gahlmann and Doerfler, 1983). These clones and subclones and purified fragments derived from them were subsequently used as ^{32}P-labeled nick-translated probes in a series of Northern blot hybridization experiments, to localize precisely the sites of homology of the low mol. wt. RNAs. The results of individual blotting experiments are summarized schematically. The graph in Figure 3 depicts a map of the site of Ad2 DNA integration and the locations of individual probes used in hybridization experiments. The designations RNA signal + and RNA signal − indicate whether or not the low mol. wt. RNA was found to be homologous to a sequence within the bounds of a certain DNA fragment. These particular data locate the cellular transcript of 300 nucleotides to a stretch of ~550 cellular nucleotides at the right site of junction between Ad2 DNA and hamster cell DNA. This RNA does not hybridize to clone pXba4 (cf. Figure 1) comprising the left site of

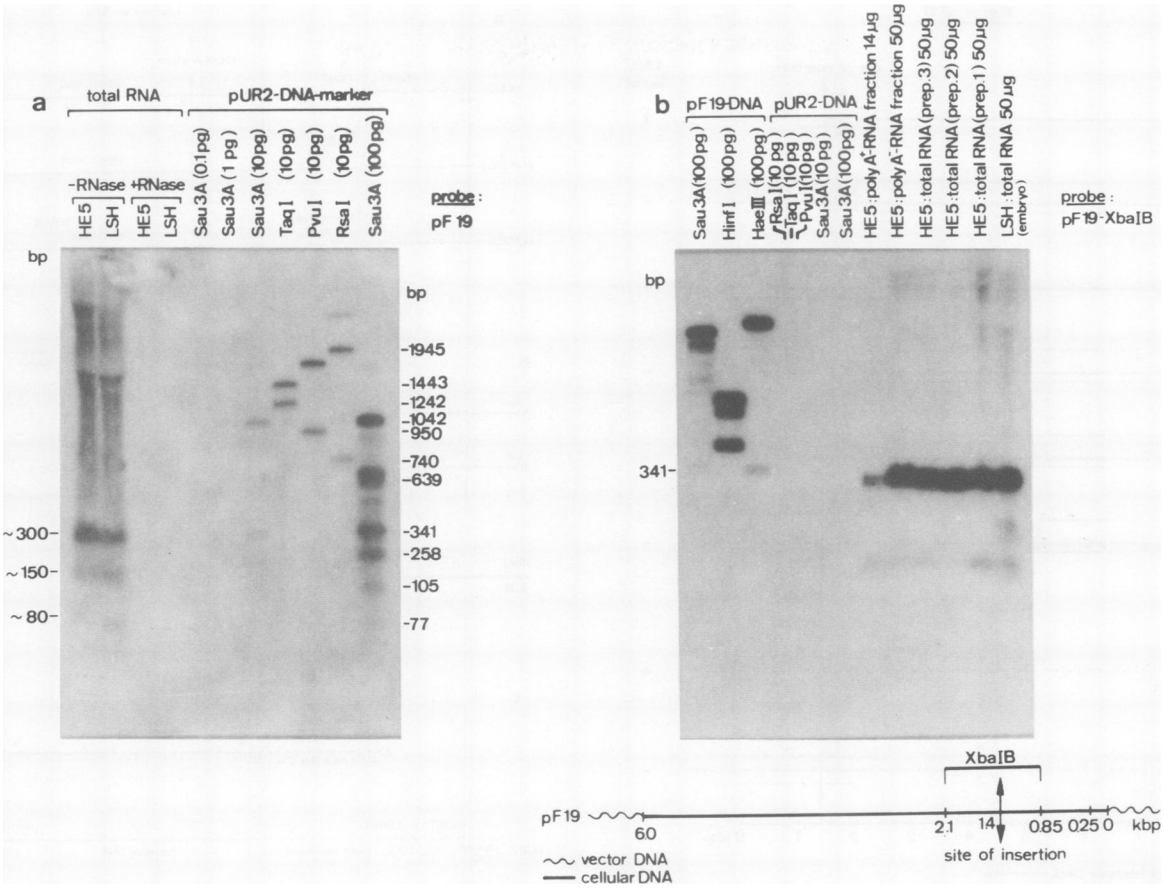

Fig. 2. Low mol. wt. RNA with homology to the site of linkage with Ad2 DNA. **(a)** Total cellular RNA from HE5 cells or from LSH hamster embryos was extracted and analyzed by Northern blot hybridization as described in the text. The cloned pre-insertion (unoccupied) site from the HE5 DNA, plasmid pF19 DNA (Gahlmann and Doerfler, 1983) was ^{32}P-labeled by nick translation and used as hybridization probe. The low mol. wt. cellular RNA signals visible are totally obliterated by treatment with pancreatic RNase. Increasing amounts (0.1 – 100 pg) of plasmid vector pUR2 DNA (Rüther, 1982) were cut with restriction endonucleases as indicated, denatured, and electrophoretically separated. The fragment signals generated served as size markers as well as to calculate approximate copy numbers per cell of the low mol. wt. RNA. The autoradiogram of a Northern blot is presented. The numbers designate RNA or DNA lengths in nucleotides. We have attempted to quantitate the low mol. wt. RNA. The intracellular RNA was extracted from 3 x 10^6 cells to yield ~50 µg of RNA which was used for gel electrophoresis. The intensity of the RNA signal in the autoradiogram shown corresponds approximately to the intensity of the 341 or the 258 nucleotide *Sau*3AI fragment of comparable length in the experiment in which 100 pg of plasmid DNA had been cleaved and electrophoretically fractionated. The total length of the pUR2 plasmid corresponds to 2685 nucleotide pairs, hence a 258 nucleotide fragment amounts to ~10 pg. One mol (equivalent to 6 x 10^{23} molecules) of single-stranded DNA of 300 nucleotides equals ~10^{17} pg. Hence 10 pg corresponds to 6 x 10^7 molecules. Assuming that the single-stranded DNA marker molecules and RNA hybridize with about equal efficiency to the pF19 probe DNA, one can assume 6 x 10^7 molecules to be derived from 3 x 10^6 cells (see above). Hence there are, on average, ~20 low mol. wt. RNA molecules per cell. **(b)** Low mol. wt. cellular RNA is not polyadenylated. RNA preparations in amounts and from sources as indicated were electrophoretically fractionated on a 1.5% agarose gel containing 2.2 M formaldehyde. Denatured DNA fragments from sources as indicated were used as size markers. After electrophoresis, DNA and RNA fragments were transferred to a nitrocellulose filter by blotting. The *Xba*IB fragment (see map at bottom) of the pF19 clone DNA was ^{32}P-labeled by nick translation and was used as probe. An autoradiogram of the filter is shown. The number 341 designates fragment size in nucleotides.

junction (Figure 3). Since the left and right terminal nucleotide sequences of Ad2 DNA are identical and are almost completely preserved in clone pXba4, it is unlikely that the transcript crosses the site of linkage into Ad2 DNA. It is important to emphasize that the mapping data for the low mol. wt. RNAs do not permit us to decide whether these RNAs are transcribed from the site of junction or from the corresponding unoccupied site.

The mapping was further refined by using subclones (Figure 4b, c) of the cellular DNA segment in bacteriophage M13. Subclones of the cellular *Eco*RI fragment, clone pF19 (cf. Figure 1a), containing the unoccupied cellular site of integration, were generated by using plasmid pUR250. Plasmids pUXP2 and pUXP7 contain *Xba*I subfragments of the junction clone as indicated in Figure 4a. The bracketed sequence between the *Hpa*I site (1.0 kbp) and the site of insertion (1.4 kbp) (Figure 4a) contains the DNA sequence homologous to the low mol. wt. RNA as documented by the data summarized in Figure 3 and by using the M13 clones as probes (Figure 4b, c). M13 clones of either polarity were used to delineate further the map locations and the polarity of the low mol. wt. RNAs. By using appropriate M13 primers, the minus strands of the cloned DNAs were synthesized and ^{32}P-labeled by incorporating α-^{32}P-labeled dNTPs. These probes were hybridized on Northern blots to total intracellular RNA from HE5 cells or from LSH hamster embryo cells. The table included in Figure 4b shows the results of these hybridization experiments in which M13 subclones of pF19 were used. The low mol. wt. cellular RNA is homologous to the DNA sequence to the left of the *Hpa*I (1.0 kb) site. The polarity of transcription of this RNA is from right to left relative to the map shown in Figure 4b. Figure 4c depicts maps of the right and left sites of junction and clones p24 and pXba4 derived from them. By using subclones of plasmids p24 and pXba4

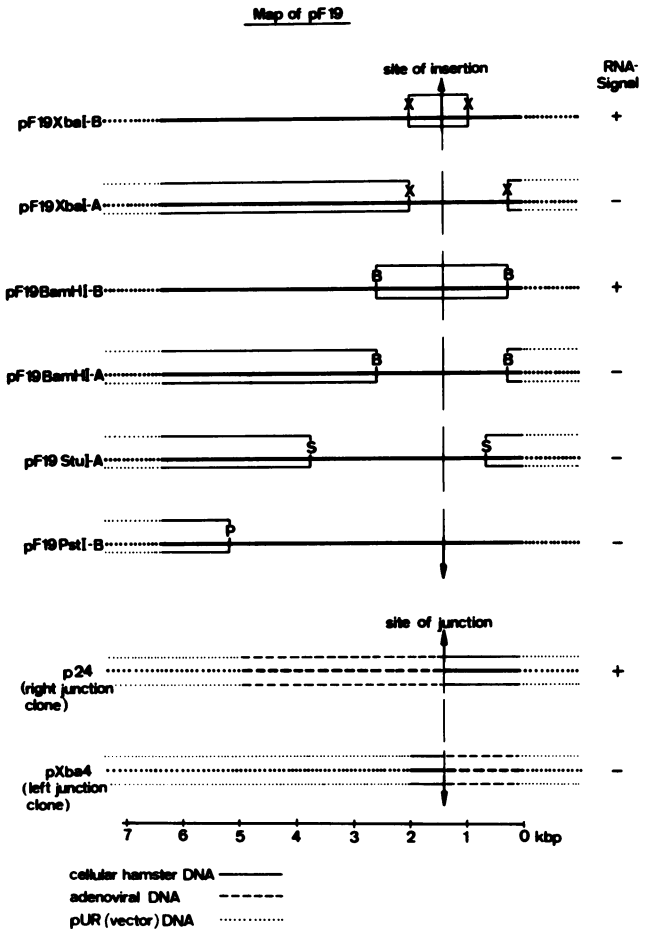

Fig. 3. Mapping of low mol. wt. cellular RNA at the site of insertion of Ad2 DNA into hamster cell DNA. Total RNA from hamster cells was fractionated by electrophoresis on 1.5% agarose gels containing 2.2 M formaldehyde. The RNA was subsequently blotted and hybridized to DNA probes as indicated. The probes were ^{32}P-labeled by nick translation. The results of several hybridization experiments are schematically summarized. The solid line boxes designate the map locations of individual *Xba*I, *Bam*HI, *Stu*I and *Pst*I subfragments of the pre-insertion clone pF19 (cf. Figure 1) containing the hamster cell DNA from the unoccupied site in HE5 cells. In some experiments, junction clones p24 (right junction) and pXba4 (left junction) containing cellular DNA and Ad2 DNA (cf. Figures 1, 4c) were also used as probes. When the DNA probe used generated an RNA signal, a + sign indicates that finding. In this way, the map location of the low mol. wt. cellular RNA is delimited. The scale at the bottom refers to the total length of the pF19 pre-insertion clone.

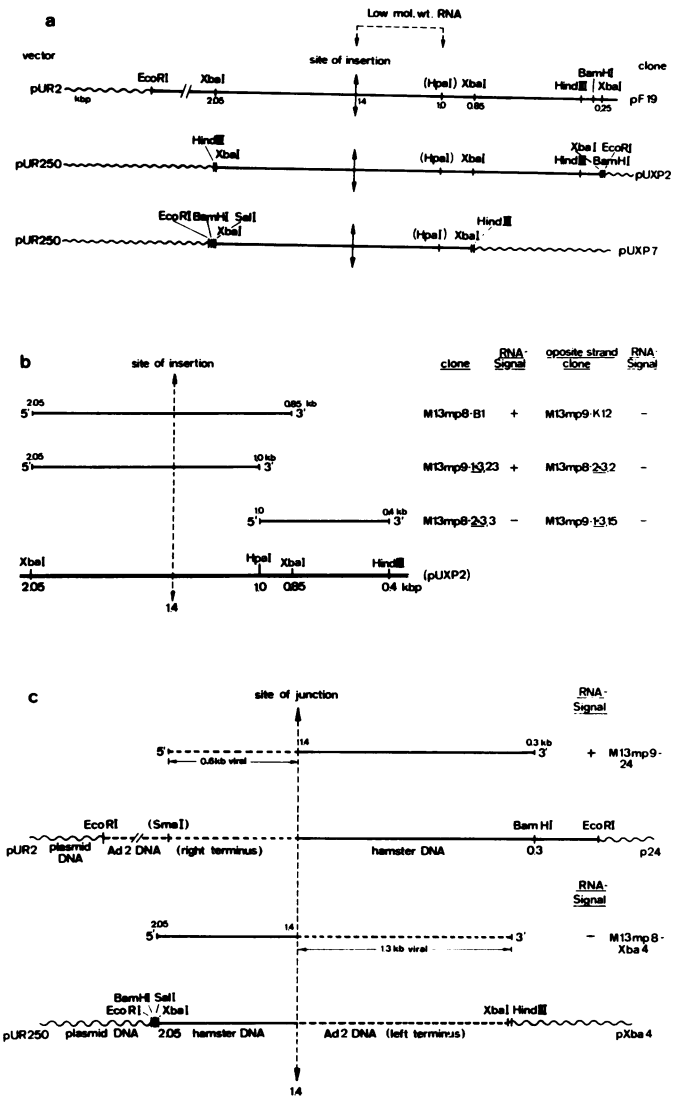

Fig. 4. Mapping of the low mol. wt. cellular RNA and determination of its polarity relative to the cellular insertion site. As described in the text, the mapping procedure was further refined by using subclones from the pF19 pre-insertion site as hybridization probes to map the location of the low mol. wt. cellular RNA. **(a)** Restriction maps of the pUXP2 and pUXP7 subclones in plasmid pUR250 (Rüther *et al.*, 1981). **(b)** Results of hybridization experiments using M13mp8 and M13mp9 clones as indicated. These clones were derived from the plasmids containing cellular DNA of the pre-insertion site. The kbp scale refers to the map position of individual subclones on the pF19 pre-insertion clone (Figure 1). The DNA fragments of indicated lengths have been cloned in suitable sites of the M13mp8 or M13mp9 vector. The table insert summarizes the results of hybridization experiments in which individual clones were used as probes. The clones M13mp8-B1, M13mp9-*1*+*3*,23 and M13mp8-*2*+*3*,3 contain the DNA strand which is shown in the figure. The clones M13mp9-K12, M13mp8-*2*+*3*,2 and M13mp9-*1*+*3*,15 contain the opposite strand. **(c)** Results of hybridization experiments using M13mp8 and M13mp9 clones derived from the right or left junction fragment as indicated.

(Figures 1, 4c) in phage M13 DNA, it was shown that all small RNAs reveal homologies to cellular DNA from map units 1.0 up to 1.4 (Figure 4c). Thus, the RNA-homologous region can be narrowed to ~400 nucleotides. It cannot be ruled out that the coding region could extend for a few nucleotides outside the limits indicated.

Occurrence of low mol. wt. cellular RNA

Cytoplasmic and nuclear RNAs were prepared from HE5 cells and were analyzed by Northern blot hybridization for the presence of the low mol. wt. cellular RNA. The ^{32}P-labeled clone pUXP7 (Figure 4a) was used as hybridization probe. The data (not presented) show the 300-nucleotide RNA to be represented mainly in the cytoplasmic RNA fraction.

The biological function of the low mol. wt. RNA is unknown. RNA molecules of identical sizes were, however, present in all major organ systems of normal LSH hamsters, perhaps with the exception of the brain (Figure 5a). The same RNA could also be detected in BHK21 hamster cells, subline B3, in the Ad12-transformed hamster cell line T637 and in LM mouse cells. Homologous RNA populations were not apparent in human HeLa cells, in oocytes from *Xenopus laevis* or in *Spodoptera frugiperda* insect cells (Figure 5b).

The low mol. wt. cellular RNA is probably non-coding

Figure 6 presents the nucleotide sequence of the cellular DNA

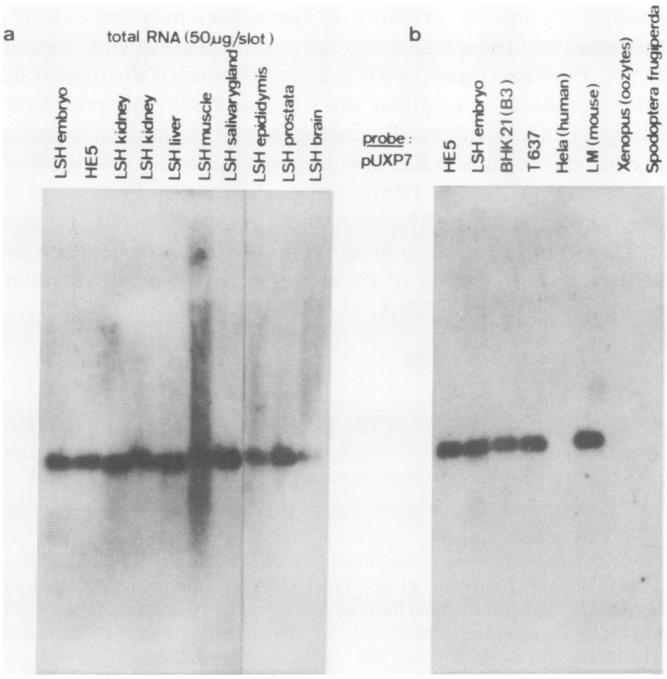

Fig. 5. Organ and species specificity of the low mol.wt. cellular RNA. Sources of the RNA analyzed have been indicated. Experimental conditions were similar to the ones described in the legends to Figure 2a and b. Plasmid pUXP7 DNA was used as hybridization probe.

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  1- 50  ACAAATCTGA AGATTAGAGT TTGATTAACA TTAGTCTACA CAAAATGCTG
 51-100  TATTTTATAA GCCTTAATAA ATTTCAAATT CAGGTTAGTT TTATTAGTAT
101-150  ATATATTTTG GGATTGCATT AAGTCTTAAC ATTTAACTAC TAGAATACAA
151-200  AAAAATGAAA GTAAATCTAG AAGGCTGTAA TTAGGATTGT TTAAAATTCT
201-250  TGGGATAACA GTAAAGCAIG IIIIGIAGIA GGIAAIGAIA AIIAAIIIIA
251-300  GGGGIGIIIA IGGGIIIAGG AGGIIAAIIG IIAGIIIGIA GIIIAAGAIA
301-350  AAIIIAAGGA AGIIAACGII AAAGTGTGAG AAAAACAAAG TAACTGTTTC
351-400  AAAAAGTGGA AGGAACTAAT TGAAGTTTGT GGGGTGTTTT TTGCCATGAG
401-450  TCCCTTGACA AGTTTTCTTT AAGATACTAC AGCTGTGACC CCACTGTACA
451-500  AATGTGGCTG GTCATAGTAT GTTAGCTACC TAGGGTCCAC CATCCCTGTG
501-550  TGCGCCCCCA CTCCCTGCTG TAGTGAGTGG GTAGAATTTA GATTTTATAA
551-600  CAGCTGGAGG CTGAGACAGG AGGACCCTGA GTTTGAGTTT AACCTGGGTT
601-650  ATGTAGCAAG ATTTTATCCA AAACAGAACA AAAACAACTT TTTTCTAATT
651-700  CAGAAATCTA TGCTCTTAAA AATGAATATT CTTCATGAAT TTAGTTTTTA
701-750  CTTAGACAAT AGATGAGAGA IAAGCIGIII IAGCICCAII CCCAIAAIGA
751-800  CIACCCCACC AIGAAAIAGA GAGCAAAGGG GAIIIIAGIA GIAAIAAAAI
801-840  GAIGGICAGG CAGCAIIAIG CTTAATCTGT GCTGTTCATT
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Fig. 6. Nucleotide sequence of the cellular DNA sequence homologous to the low mol. wt. cellular RNA. Part of this sequence has been published previously (Gahlmann and Doerfler, 1983). The site of insertion of Ad2 DNA is indicated by a double-headed verticle arrow. The sequence underlined represents the most likely sequence of the low mol. wt. cellular RNA. The sequence of the non-coding DNA strand is reproduced which corresponds to the RNA sequence itself. Regions designated by broken lines are those into which the low mol. wt. RNA might extend. The sequence of the non-coding DNA is shown in the 5'-3' direction. In order to preserve that conventional way of presentation, the site of Ad2 DNA insertion has been positioned to the right of the cellular sequence homologous to the low mol. wt. RNA. The orientation has thus been reversed compared with that shown in previous figures.

segment homologous to the low mol. wt. RNA. This nucleotide sequence was subjected to extensive computer-aided analyses. (i) Open reading frames >18 consecutive amino acids are not detectable. There are no TATA signals or sequence motifs detectable that have been recognized as characteristic for promoter regions of a gene. Thus, it is very unlikely that the low mol. wt. cellular RNA can code for a larger polypeptide. (ii) As far as tested, the low mol. wt. cellular RNA has no apparent sequence homologies with any of the known low mol. wt. RNAs, such as sRNAs, tRNAs or small rRNAs of eukaryotic origin. (iii) Striking secondary structures cannot be detected in the cellular DNA sequence.

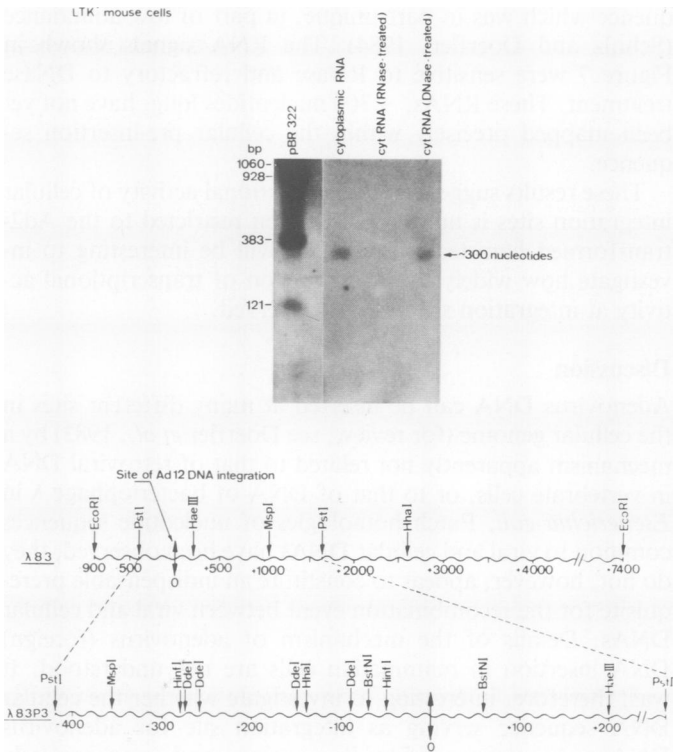

Fig. 7. Cytoplasmic RNA from mouse Ltk⁻ cells with homologies to the cellular pre-insertion site of Ad12 DNA. This pre-insertion site corresponds to the integration site in the CBA-12-1-T mouse tumor. Cytoplasmic RNA from mouse Ltk⁻ cells was isolated as described (Scott *et al.*, 1983). RNA preparations (50 µg) were electrophoresed on a 1.5% agarose gel containing 2.2 M formaldehyde and were blotted to a nitrocellulose filter as described (Schirm and Doerfler, 1981). As indicated, 50 µg of RNA was treated either with pancreatic RNase A (20 µg/ml) or with DNase (BRL), and these preparations were also electrophoresed. Plasmid pBR322 DNA was cut with *Bst*NI, denatured and co-electrophoresed (0.1 ng) as size marker. The 2.0-kb *Pst*I insert of mouse cell DNA in clone λ 8.3P3 (Schulz and Doerfler, 1984) was excised, purified (Materials and methods), ^{32}P-labeled by nick translation (Rigby *et al.*, 1977) and used as hybridization probe. The pBR322 marker strip was separately hybridized to ^{32}P-labeled pBR322 DNA and was reattached to the main part of the filter before autoradiography. The map location of the DNA probe was also indicated.

Low mol. wt. cellular RNA homologous to the cellular site of insertion of Ad12 DNA into mouse cell DNA

The site of linkage between the left end of Ad12 DNA and mouse cell DNA in the Ad12-induced tumor CBA-12-1-T was cloned and sequenced (Schulz and Doerfler, 1984). The pre-insertion sequence from normal CBA/J cells was also cloned and sequenced. A 2.0-kb subcloned segment of this pre-insertion sequence, which corresponded to the integration site of Ad12 DNA, was used to probe cytoplasmic RNA from mouse Ltk⁻ cells for homologous sequences. Similar experiments were performed with RNA from various organs of CBA/J or C57/B mice. The data presented in Figure 7 demonstrated that the cytoplasmic RNA from mouse Ltk⁻ cells exhibited homologies to the cellular pre-insertion se-

quence which was in part unique, in part of low abundance (Schulz and Doerfler, 1984). The RNA signals shown in Figure 7 were sensitive to RNase and refractory to DNase treatment. These RNAs, ~300 nucleotides long, have not yet been mapped precisely within the cellular pre-insertion sequence.

These results suggest that transcriptional activity of cellular integration sites is not an observation restricted to the Ad2-transformed hamster line HE5. It will be interesting to investigate how widely the phenomenon of transcriptional activity at integration sites can be observed.

Discussion

Adenovirus DNA can be inserted at many different sites in the cellular genome (for review, see Doerfler *et al.*, 1983) by a mechanism apparently not related to that of retroviral DNA in vertebrate cells, or to that of DNA of bacteriophage λ in *Escherichia coli*. Patch homologies of nucleotide sequences common to viral and cellular DNAs have been observed, they do not, however, appear to constitute an indispensable prerequisite for the recombination event between viral and cellular DNAs. Details of the mechanism of adenovirus (foreign) DNA insertion in mammalian cells are not understood. It was, therefore, interesting to investigate whether the cellular DNA sequence serving as integration site for adenovirus DNA was transcriptionally active and consequently predisposed for foreign DNA insertion. We chose the Ad2-transformed cell line HE5 which has a relatively simple pattern of viral DNA integration and which has consequently been studied in greatest detail.

Low mol. wt. RNAs, measuring ~300 nucleotides, have been found with homologies to the hamster cell DNA sequence abutting the right terminus of the integrated Ad2 genome. It is impossible to decide whether these RNA molecules were transcribed from the actual site of adenovirus DNA integration or from the allelic unoccupied cellular DNA sequence corresponding to the insertion site. These cellular RNA sequences are not polyadenylated, are predominantly cytoplasmic, and are expressed in the Ad2-transformed hamster cell line HE5 as well as in all organs of normal LSH hamsters, in embryonal hamster tissue, in BHK21 cells and in Ad12-transformed T637 cells; they are also detected in mouse cells, but not in human, amphibian or insect cells. There is no evidence that these short RNAs can be translated into polypeptides, the longest open reading frame in the relevant DNA sequence could code for a polypeptide of only 18 amino acids. We have also searched in mouse cells for RNA with homologies to the site of integration of Ad12 DNA in the Ad12-induced mouse tumor CBA-12-1-T (Schulz and Doerfler, 1984), and similar low mol. wt. RNAs have been detected which exhibit homologies to the site of Ad12 DNA insertion.

The biological functions of the low mol. wt. cellular RNAs are not known. The RNA is probably not related to the transformed state of cells, since similar RNAs are also found in normal cells and tissues. The low mol. wt. hamster cell RNA has no similarities to other known eukaryotic low mol. wt. RNAs. With respect to the relationship of cellular transcripts to the integration of foreign (adenovirus) DNA, it is interesting to note that the site of integration of adenovirus DNA, or its unoccupied allelic sequence, or both sequences are transcriptionally active and perhaps for that reason predisposed for recombination with foreign DNA. The specific chromatin structure at these sites may increase the propensity of these sequences for recombination with foreign DNA. Perhaps transcriptional activity is one of the important preconditions for a cellular site to facilitate the integration of foreign DNA. Evidence for increased recombination in active regions of chromatin has also been attained in yeast (Storb *et al.*, 1981), in murine lymphocytes (Nasmyth, 1982), and in the chicken system (Schubach and Groudine, 1984). Further work will be required to investigate relationships between the transcriptional activity of mammalian chromatin and the integration of foreign DNA.

Materials and methods

LSH inbred hamsters

Mesocricetus auratus pregnant females were obtained from Charles River Breeding Laboratories, Inc., Wilmington, MA. RNA was extracted from 14-day old embryos. Individual organs were prepared from 15–16 week old animals.

Cells and virus

The following cells were grown in culture using Dulbecco's modification of Eagle medium (Bablanian *et al.*, 1965) supplemented with 10% fetal calf serum: the Ad2-transformed hamster cell line HE5 (Cook and Lewis, 1979), the Ad12-transformed hamster cell line T637 (Sutter *et al.*, 1978), human HeLa cells and mouse LM and Ltk$^-$ cells. *S. frugiperda* insect cells were propagated in synthetic medium (Gardiner and Stockdale, 1975) containing 0.26% tryptose phosphate broth as described elsewhere (Tjia *et al.*, 1979). All media were supplemented with 10% fetal calf serum. Oocytes from *X. laevis* were activated and prepared as described (Kressmann and Birnstiel, 1980; Langner *et al.*, 1984).

General methods

Many of the cloning methods used have been described earlier (Gahlmann *et al.*, 1983). The techniques for DNA-DNA hybridization (Wahl *et al.*, 1979), nick translation of DNA and DNA fragments (Rigby *et al.*, 1977), Southern blotting, (Southern, 1975) and separation of RNA molecules on agarose-formaldehyde gels (Schirm and Doerfler, 1981) have been previously outlined.

Some of the DNA fragments were subcloned in bacteriophage M13mp8 or M13mp9 (Messing and Vieira, 1982). Cloned DNA fragments which were used as hybridization probes were ^{32}P-labeled as described (Hu and Messing, 1982).

The origin of the λ 8.3 P3 clone was described (Schulz and Doerfler, 1984). This clone contained the pre-insertion DNA sequence from CBA/J mouse cells. Ad12 DNA was integrated into this sequence in the Ad12-induced mouse tumor CBA-12-1-T. The 2.0-kb insert of this clone (cf. Figure 7) was excised with *Pst*I, three times purified by agarose gel electrophoresis and eluted from the gel by the DEAE-membrane filter (NA 45) method (Dretzen *et al.*, 1981).

Determination of DNA nucleotide sequences

The Maxam-Gilbert (1977, 1980) and the dideoxynucleotide sequencing methods (Sanger *et al.*, 1977) were used as described.

Extraction and purification of RNA

Preparation of total intracellular RNA. RNA was extracted from cells that had grown to half-confluency in monolayers. The method of Auffray and Rougeon (1980) was used. The same method was employed to extract total RNA from hamster organs or embryos which had been previously frozen in liquid N_2.

Preparation of total RNA from hamster organs. Liquid N_2-frozen organs were homogenized at 0°C in at least 4 volumes of a solution of 5 M guanidinium thiocyanate, 0.1% SDS, 0.05 M Tris-HCl, pH 7.5, 5% 2-mercaptoethanol. The method of Chirgwin *et al.* (1979) and Bernstein *et al.* (1983) was followed.

Extraction of cytoplasmic and nuclear RNA. Cells grown to half-confluent monolayers were resuspended in 1 ml of 0.01 M Tris-HCl, pH 8.1, 1 mM EDTA, 0.1 M NaCl at 0°C. This solution was adjusted to 5 mM $MgCl_2$ and 0.5% Nonidet P-40 (Scott *et al.*, 1983). The nuclei were pelleted at 9500 r.p.m. and 4°C for 2 min. The cytoplasmic supernatant was adjusted to 0.5% SDS, 0.025 M EDTA and was extracted with Tris-HCl-saturated phenol at 65°C for 10 min. The aqueous phase was extracted with a phenol:chloroform:isoamyl alcohol (25:24:1 by vol.) mixture and a chloroform:isoamyl alcohol (24:1 v/v) mixture.

The nuclear RNA was extracted from nuclei as described above. The method of Scott *et al.* (1983) was also used to prepare the total cytoplasmic

RNA from various organs of 5–6 week old CBA/J or C57/B mice.

Polyadenylated mRNA

This was selected by adsorption to oligo(dT)-Sepharose as described elsewhere (Aviv and Leder, 1972).

Computer analyses

The computer analyses of nucleotide sequences were performed using a VAX11/780 computer. The following programs were used: BestFit, Map, MapPlot, DotMatrix (Devereux *et al.*, 1984), and Structure (Kurt Stüber, personal communication).

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