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Patch homologies and the integration of adenovirus DNA in mammalian cells

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The hamster cell line HE5 has been derived from primary hamster embryo cells by transformation with human adenovirus type 2 (Ad2). Each cell contains 2 – 3 copies of Ad2 DNA inserted into host DNA at apparently identical sites. The site of the junction between the right terminus of Ad2 DNA and hamster cell DNA was cloned and sequenced. The five right terminal nucleotides of Ad2 DNA were deleted. The unoccupied cellular DNA sequence in cell line HE5, corresponding to the site of the junction between Ad2 and hamster cell DNA, was also cloned; 120 – 130 nucleotides in the cellular DNA were found to be identical to the cellular DNA sequence in the cloned junction DNA fragment, up to the site of the junction. The unoccupied and the occupied cellular DNAs and the adjacent viral DNA exhibited a few short nucleotide homologies. Patch homologies ranging in length from dodeca- to octanucleotides were detected by computer analyses at locations more remote from the junction site. When the right terminal nucleotide sequence of Ad2 DNA was matched to randomly selected sequences of 401 nucleotides from vertebrate or prokaryotic DNA, similar homologies were observed. It is likely that foreign (viral) DNA can be inserted *via* short sequence homologies at many different sites of cellular DNA.

Key words: adenovirus transformed cells/junction sites/nucleotide sequence

Introduction

The uptake and fixation of foreign DNA by mammalian cells can be studied in great detail by using viral DNA as a model. We have analyzed the sites of integration of adenovirus DNA in a large number of adenovirus-transformed cells, or in tumors induced in rodents by adenovirus type 12 (Ad12) (for review, see Doerfler, 1982). Restriction enzyme analyses combined with Southern blotting (Southern, 1975) yielded patterns of integration of adenovirus DNA that were different in some 70 cell lines or tumors investigated (Sutter *et al.*, 1978; Stabel *et al.*, 1980; Vardimon and Doerfler, 1981; Kuhlmann and Doerfler, 1982). Similar results were reported by other laboratories (Visser *et al.*, 1980; Sambrook *et al.*, 1980; Green *et al.*, 1981). In the Ad12-induced tumor line CLAC3, we found remarkable patch-type homologies between the adjacent cellular and viral DNA sequences (Deuring *et al.*, 1981). Such patch homologies were also reported in defective SV40 genomes (Gutai and Nathans, 1978) and in SV40-transformed cells (Stringer, 1981, 1982). The sites of the junctions between viral and cellular DNA in a number of SV40- and adenovirus-transformed cell lines have been sequenced (Sambrook *et al.*, 1980; Deuring *et al.*, 1981; Stringer, 1981, 1982; Westin *et al.*, 1982).

We have sequenced the site of the junction between the right terminus of Ad2 DNA and hamster cell DNA in HE5 cells. A total of 282 nucleotides of viral and 401 nucleotides of cellular origin was analysed. The right terminal five nucleotides of Ad2 DNA were deleted, the viral nucleotide sequence was otherwise unaltered. The cellular DNA sequence up to the junction site was also unaltered when compared with the cellular sequence of the unoccupied site in HE5 DNA. Computer analyses revealed numerous patch-type homologies between cellular and viral DNA sequences and between Ad2 DNA and randomly selected eukaryotic or prokaryotic DNA sequences.

Results and Discussion

Cloning and recloning do not alter the junction site between hamster cell DNA and the right terminus of Ad2 DNA

It was necessary to ascertain that, upon molecular cloning of the junction site, DNA sequences were not altered. DNAs from cell line HE5, from the pUR2 clone (see Materials and methods), and from Ad2 were cleaved with *Eco*RI, and the fragments were compared by blotting using ^{32}P-labeled Ad2 DNA as probe. The data (not shown) indicated that the viral-cellular DNA junction fragment was not altered by deletions or rearrangements upon cloning and recloning.

Restriction map of the cloned DNA fragment

A detailed restriction map of the cloned DNA fragment was established (Figure 1) using a number of restriction endonucleases and the method of Smith and Birnstiel (1976). The fragment containing the junction could always be identified by its off-size position relative to Ad2 DNA fragments used as internal size standards.

Distribution of clone-specific cellular DNA fragments in DNA from normal hamster cells, from line HE5, from other transformed lines, and from some vertebrate species

A DNA fragment consisting exclusively of cellular DNA sequences (bar in Figure 1) was excised from the cloned sequence and used as hybridization probe (Figure 2) with *Eco*RI-cut cellular DNA from various vertebrate sources. The ^{32}P-labeled fragment hybridized to one band of identical size in all DNA preparations tested, except in human KB cell DNA which lacked any homology. A second band was apparent in the DNA of all Ad2-transformed or normal hamster cells (Figure 2) and probably corresponded to the unoccupied cellular site from the second chromosome. This fragment was recloned from line HE5 and sequenced also (see below). The data also showed that the size of the cloned DNA fragment (Figure 2, track f) was still identical to the same fragment in the DNA from HE5 (Figure 2, track g) after cloning. Thus, there was no evidence for deletions or rearrangements in the DNA fragment during molecular cloning.

Nucleotide sequence at the junction site

At the site of the junction between the right end of Ad2 DNA and hamster cell DNA, the sequence of 282 nucleotides

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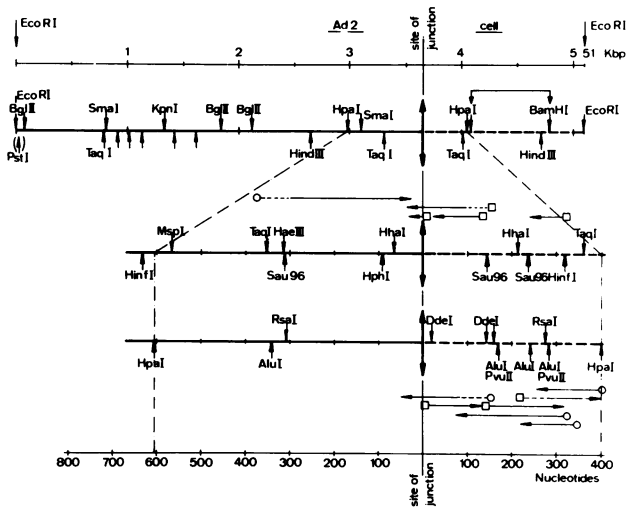

Fig. 1. Restriction maps of the cloned DNA junction fragment. Maps of the entire 5.1-kbp fragment cloned in the λgtWES·λB and recloned in the pUR2 vector were determined. Ad2 DNA is represented by solid lines, cellular DNA by broken lines. The bar with the downward vertical arrows (⌐¬) brackets the hamster cell *HpaI/Bam*HI DNA fragment that was excised and used as hybridization probe in the experiment described in Figure 2. The site of junction between viral and cellular DNA is marked by bidirectional vertical arrows. The map of the 1.1-kbp *Hpa*I DNA fragment encompassing the junction site is presented as an enlargement. The horizontal arrows designate map positions of DNA fragments near the site of the junction which were used in sequencing experiments. The direction of nucleotide sequencing is also indicated. Arrows attached to a circular symbol (○) represent DNA fragments that were excised from the gel and ^{32}P-labeled at the 5′ termini using [γ-^{32}P]ATP and polynucleotide kinase. Arrows with a square symbol (□) correspond to DNA fragments ^{32}P-labeled at the 3′ termini using [α-^{32}P]dNTPs and the Klenow fragment of *E. coli* DNA polymerase I. DNA size scales in kbp (top) or in nucleotides (bottom) are also shown. Details of each sequencing experiment are available on request.

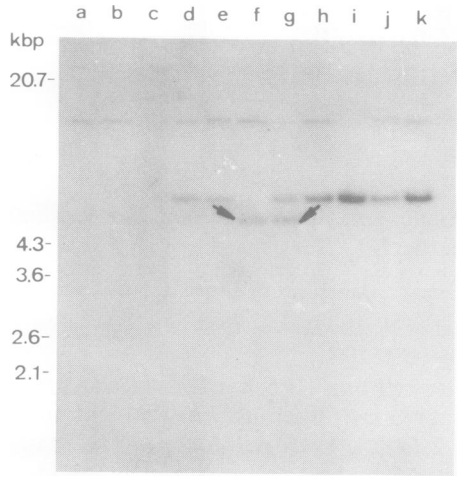

Fig. 2. Homology of a hamster cell DNA fragment (*HpaI/Bam*HI) from clone pUR2 to cellular DNA from salmon sperm, rat, hamster, and human cells. DNA (10 μg) was cleaved with *Eco*RI, the fragments were separated by electrophoresis on horizontal 0.5% agarose slab gels and blotted to nitrocellulose filters: **(a)** salmon sperm DNA containing two genome equivalents per cell of Ad2 DNA; **(b)** DNA from rat embryo cells; **(c)** DNA from human KB cells; **(d)** DNA from the BHK21 hamster line B3; **(e)** DNA from primary LSH hamster embryo cells; **(f)** salmon sperm DNA and pUR2 HE5 clone DNA; **(g)** DNA from the Ad2-transformed hamster line HE5; **(h)**, **(i)**, **(j)**, and **(k)** DNA from the Ad2-transformed lines HE4, HE3, HE2, and HE1, respectively (c.f. Vardimon and Doerfler, 1981). A cellular *HpaI/Bam*HI DNA fragment from the pUR2 insert as indicated by a bracket in the scheme in Figure 1 was excised from the cloned DNA by restriction endonucleases, purified by electrophoresis on a 4% polyacrylamide slab gel, and eluted. Subsequently, this DNA fragment was ^{32}P-labeled by nick-translation (Rigby *et al.*, 1975) and used as hybridization probe. After DNA-DNA hybridization, specific DNA fragments were visualized by autoradiography. The sizes of the *Eco*RI fragments of Ad2 DNA (not shown) used as markers are indicated at the left margin.

in viral DNA and of 401 nucleotides in cellular DNA was determined (Figure 3). In the Ad2 sequence, the last five nucleotides have been deleted. The remainder of the viral DNA sequence in the clone was identical with the sequence of virion DNA (Roberts *et al.*, 1982). It was interesting to note that in Ad12-induced tumor lines CLAC3 (Deuring *et al.*, 1981) and CLAC1 (Stabel and Doerfler, unpublished data) 45 and 174 nucleotides of viral DNA, respectively, had been deleted at the sites of the junction. Thus, whatever mechanisms viral DNA insertion might entail, deletions of short stretches of viral DNA appear to be a common feature.

Computer analyses of the nucleotide sequence

At the immediate site of the junction, homologies between the viral and cellular DNA sequences did not exist. Since patch homologies between viral and cellular DNA had been seen earlier (Deuring *et al.*, 1981), we carried out a computer search. There was indeed a surprisingly large number of such patch-type homologies ranging in length from dodeca- down to octanucleotides (map in Figure 4). Shorter sequences that occurred in abundance were not recorded. The longer patch homologies were also observed, though rarely, in 400 000 nucleotides randomly selected from prokaryotic or eukaryotic DNA sequences. The distribution of patches between viral and cellular DNA sequences showed no apparent symmetry (Figure 4). In some instances the patches tended to cluster, particularly in the cellular DNA sequence. The patches were rich in A-T base pairs (legend to Figure 4). We also searched for patch-type homologies with the right end of Ad2 DNA among randomly selected eukaryotic or prokaryotic DNA sequences. There were numerous patches ranging from octa- to dodecanucleotides even in completely unrelated cellular DNA sequences and in prokaryotic DNA (Table I). The actual sequence strings were different in each case, and they were different from the patch sequences detected in the hamster cell DNA sequence.

It is very unlikely that the patch homologies could be the consequence of extensive sequence rearrangements during or after the integration event or a cloning artefact. Except for five nucleotides missing at the terminus, the viral part of the junction sequence is unaltered. Moreover, the cellular sequence up to the junction site has been completely preserved (Figure 5, see below). Patch-type sequence homologies between the right part of Ad2 DNA and randomly selected sequences from vertebrates or from prokaryotes are rather common (Table I). It is therefore possible that insertion of foreign (viral) DNA is directed by short sequence homologies. The nucleotide sequences in the macroenvironments at the sites of insertion of adenovirus genomes in hamster or mouse cell DNA are not specific (Doerfler, 1982). The apparent abundance of patch-type sequence homologies is consistent with many possible sites of recombination and with the notion that short sequence homologies serve as signals to direct the insertion event.

Nucleotide sequence of the unoccupied cellular site in cell line HE5

The unoccupied cellular DNA fragment was cloned from cell line HE5 as described in the legend to Figure 5. The

nucleotide sequence of the cellular DNA fragment corresponding to the site of the junction was determined (Figure 5). The cellular DNA sequence from the first *Dde*I site (Figure 1, line 4) to the site of the junction and the sequence between the first and second *Dde*I sites (Figure 1) were identical to those found in the junction clone (Figure 3). The corresponding sequences in the original cellular DNA (Figure 5, bottom line) and the Ad2 DNA (top line) show short homologies comprising maximally tetranucleotides. Thus, it is reasonable to pur-

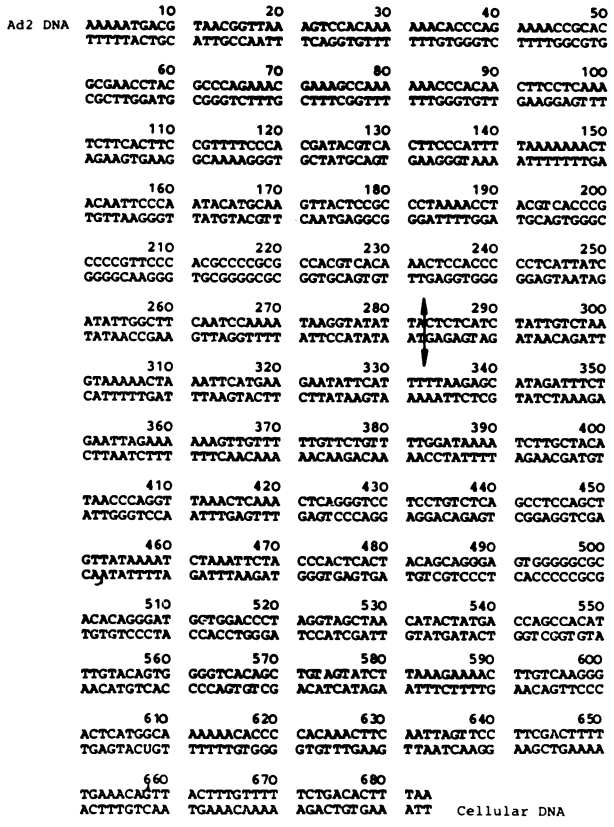

Fig. 3. The nucleotide sequence at the junction site between the right terminus of Ad2 DNA and hamster cell DNA in cell line HE5. The junction site has been indicated by a vertical bidirectional arrow.

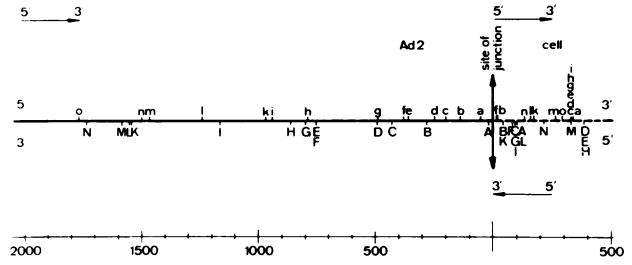

Fig. 4. Schematic representation of patch homologies between Ad2 DNA and hamster DNA sequences around the junction site in cell line HE5. The scale at the bottom indicates the number of nucleotides in either direction from the junction site. The letters refer to individual nucleotide patches as indicated. Capital letters designate homologies in the right-to-left direction, lower case symbols homologies in the opposite direction. The junction site is denoted by a bidirectional arrow. The following homologies have been observed:

A: ATCCAAAA	(8)	**a**: CACAAACT	(8)
B: AAAAATGA	(8)	**b**: AAAAACTA	(8)
C: CAGAACAA	(8)	**c**: CAAAAAAC	(8)
D: AGAAAAACA	(9)	**d**: CAAAAAACACCC	(12)
E: AAAAACAAC	(9)	**e**: AAAAAACACC	(10)
F: GAAAAACAA	(9)	**f**: AGTAAAAA	(8)
G: AACAAAAA	(8)	**g**: AAAAACAC	(8)
H: CAGAAAAA	(8)	**h**: AAAAAACA	(8)
I: AACAAAAA	(8)	**i**: GCAAAAAA	(8)
K: AAAATGAA	(8)	**k**: TATAAAAT	(8)
L: TTATCCAAAAC	(11)	**l**: TCAGCCTC	(8)
M: TGTTTTTT	(8)	**m**: CATTTGTA	(8)
N: GCGCCCCC	(8)	**n**: TCAAACTC	(8)
		o: TGTAGTAT	(8)

Table I. Frequency of oligonucleotides common between the right end of Ad2 DNA (*Eco*RI fragments F, D, E, and C, (l-strand)) and randomly selected prokaryotic and eukaryotic DNA sequences[a]

DNA sequence from	Length of oligonucleotide				
	8	9	10	11	12
(a) Prokaryotic organisms					
E. coli, lac y	45	9	2	–	–
E. coli, rec A	36	10	2	1	–
Phage λ, 12	39	13	3	1	–
Phage λ, rex	41	12	2	1	–
(b) Eukaryotic organisms					
HE5 hamster line, cell DNA in clone, l-strand	50	11	2	1	1
HE5 hamster line, cell DNA in clone, r-strand	54	11	2	2	–
Human preproinsulin	52	11	–	–	–
Human interferon 1B	47	12	10	–	1
Human ϵ globin	43	10	1	–	–
Human Ig, kappa chain	58	13	2	1	–
Murine β globin	38	14	1	2	1
Murine IgG	46	13	3	1	–
Chicken ovalbumin	39	11	2	–	1

[a]The prokaryotic and eukaryotic sequences screened were 401 nucleotides long.

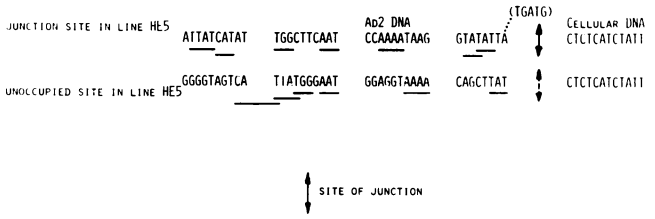

Fig. 5. Comparison of the nucleotide sequences at the junction site between Ad2 DNA and hamster cell DNA (**top**) and the corresponding nucleotide sequence of the unoccupied cellular site (**bottom**) from cell line HE5. The 5.5-kb cellular *Eco*RI DNA fragment from cell line HE5 (Figure 2, track g, second band from top) was cloned in λgtWES·λB DNA and subcloned in pUR2 DNA using the clone carrying the Ad2-cellular DNA junction fragment as ^{32}P-labeled hybridization probe. The junction fragment or the corresponding cellular DNA fragment was then excised with *Eco*RI from the λ vector, subsequently cut with *Hin*fI (c.f. Figure 1), blotted and hybridized to the cloned Ad2-cellular DNA junction fragment which was ^{32}P-labelled. The *Hin*fI fragment from the cellular DNA fragment, which corresponded to the junction fragment, was isolated by gel electrophoresis, electroeluted, and recut with *Dde*I. The *Dde*I fragment corresponding to the junction site was sequenced, and this sequence is reproduced here. The *Dde*I fragment adjacent to the right and comprising some 120 nucleotides was also sequenced (data not shown). This sequence was identical to the corresponding sequence shown in Figure 3. Short sequences of homology between Ad2 DNA and the original cellular DNA sequence are underlined.

sue the possibility that recombinations between viral and cellular DNA may be directed by short sequence homologies.

Materials and methods

Most of the methods used have been described earlier (Stabel *et al.*, 1980; Deuring *et al.*, 1981).

The Ad2-transformed hamster cell line HE5 was obtained by transformation of primary LSH hamster embryo cells with u.v.-inactivated Ad2 in culture (Johansson *et al.*, 1978; Cook and Lewis, 1979).

Molecular cloning of the junction site

The pattern of viral DNA insertion in line HE5 had been determined (Vardimon and Doerfler, 1981). From the results, the junction with cellular DNA at the right terminus of Ad2 DNA appeared suitable for molecular cloning. Experimental conditions were similar to those described previously (Deuring *et al.*, 1981). The DNA of bacteriophage λgtWES·λB was used as the cloning vector. Preselected DNA from line HE5 and λ 'arms' were mixed at a weight ratio of 1:6, and the DNA preparations were ligated at 25°C for 1 h using T4 DNA ligase. The ligated DNA was packaged *in vitro* into phage λ heads (Hohn and Murray, 1977). About $1.5 \times 10^6 - 2 \times 10^6$ plaques were screened to discover six positive plaques. The HE5 fragment from one clone was recloned into plasmid pUR2 DNA (Rüther, 1980). The unoccupied cellular DNA site from cell line HE5 was also cloned by using the cloned *Eco*RI junction DNA fragment in pUR2 as hybridization probe.

Restriction mapping and isolation of fragments

Conventional mapping methods were used employing the restriction endonucleases *Alu*I, *Bam*HI, *Bgl*II, *Dde*I, *Eco*RI, *Hae*III, *Hha*I, *Hind*III, *Hin*fI, *Hpa*I, *Hph*I, *Kpn*I, *Msp*I, *Pvu*II, *Pst*I, *Rsa*I, *Sau*96I, *Sma*I, and *Taq*I.

Determination of the nucleotide sequence

In all nucleotide sequencing work the method of Maxam and Gilbert (1980) was used. All sequences were confirmed either by overlapping sequences or by sequencing both strands of a given DNA fragment.

Computer analyses

Computer analyses of the nucleotide sequence were performed on a Control DATA Cyber-76 computer. FORTRAN IV-programs were used for all computations. Initially a search was conducted for sub-strings of eight nucleotides, later the search was extended to longer strings to find patch homologies between the right end of Ad2 DNA (Table I) and the adjacent cellular DNA or in randomly selected 400 000 nucleotides from prokaryotic or eukaryotic DNA.

Acknowledgements

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