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Nucleotide Sequence of Human Adenovirus Type 12 DNA: Comparative Functional Analysis

JÖRG SPRENGEL, BIRGIT SCHMITZ, DAGMAR HEUSS-NEITZEL,† CHRISTIANE ZOCK, AND WALTER DOERFLER*

Institute of Genetics, University of Cologne, Cologne, Germany

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A fresh inoculum of human adenovirus type 12 (Ad12) was obtained from the American Type Culture Collection and passaged once on human embryonic kidney cells, and Ad12 DNA was prepared from the first-passage yield to avoid higher passages which might have generated host-virus DNA recombinants. The 18 *Pst*I fragments of Ad12 DNA were cloned into the pBluescript KS vector, and the entire nucleotide sequence of both strands from all 18 fragments was determined by using successive oligodeoxyribonucleotide primers. Ad12 DNA extends over 34,125 nucleotide pairs, and its molecular weight is calculated to be about 22 × 10^6. The nucleotide sequence of Ad12 DNA was subjected to computer analyses that determined possible open reading of frames on the two strands, the leader sequences, the position of the virus-associated RNA coding region, possible TATA, and polyadenylation signals. The distribution of the Ad12 open reading frames was similar to that in the previously sequenced Ad2 DNA, but there were also distinct differences. Ad12 DNA has an inverted terminal redundancy of 161 nucleotides, compared with 102 nucleotides in Ad2 DNA. There were stretches of sequence identity between Ad2 and Ad12 DNAs at both termini; the overall sequence similarity between the two viral genomes ranged between 59% (polypeptide IX) and 77% (in the E2 region), with high homology also in the sequences for the adenovirus DNA polymerase.

Over a period of several decades, we have used human adenovirus type 12 (Ad12) and its genome for studies of the molecular biology of mammalian cells and the molecular mechanism of viral oncogenic transformation (see references 13, 15, and 18 for recent reviews). Adenoviruses have been very valuable models in molecular biology and oncology, and many aspects of adenovirology are still under intensive investigation.

The main topics of research on Ad12 in this laboratory have been the mechanism of Ad12 DNA integration into the mammalian genome (11, 16, 43, 44), the de novo methylation of integrated Ad12 genomes in hamster cells (29, 42), and the abortive interaction of Ad12 virions with hamster cells (12, 50, 51).

Nucleotide sequences in a number of adenovirus genomes have been determined previously and have been compared (48). The nucleotide sequences of the genomes of Ad2 (33) and Ad5 (6) have previously been completely determined. It is beyond the scope of this paper to list all the published partial sequences of all human adenoviruses. Several laboratories have contributed partial nucleotide sequences of Ad12 DNA in the past, and these sequences are summarized in Table 1.

MATERIALS AND METHODS

From a fresh Ad12 inoculum (ATCC VR-863) purchased from the American Type Culture Collection, Ad12 was propagated once on human embryonic kidney (HEK) cells, and the *Pst*I fragments of the DNA isolated from the purified virions were cloned into the pBluescript KS vector by standard procedures. The nucleotide sequences of all fragments were determined in an Applied Biosystems 373A sequencer by using appropriate 19- to 24-nucleotide-pair primers and the chain termination method (37). The nucleotide sequence of both DNA strands was determined over the entire molecule (see the primer locations in Fig. 1). The nucleotide sequence of 34,125 bp of the Ad12 genome has been communicated to the EMBL data library and will be published separately (22a). Here, we present a functional analysis of the nucleotide sequence of Ad12 DNA and comparisons with the known nucleotide sequences of some of the other human adenovirus genomes.

We have shown previously that, in productively infected human cells, Ad12 DNA can recombine with host DNA. This recombination event can generate symmetric Ad12-host DNA recombinant (SYREC) molecules (9). In one instance, the SYREC2 DNA consisted of a long inverted tandem repeat of the left terminal 2,081 bp of Ad12 DNA and of human cellular DNA (8). To avoid the potential contamination of the Ad12 nucleotide sequence with human cellular sequences, we prepared DNA from a fresh, low-passage Ad12 inoculum. All 18 *Pst*I fragments (A to R) of Ad12 DNA were subsequently cloned from this independently derived DNA preparation into the plasmid vector pBluescript KS. We have shown by *Pst*I cleavage of this DNA, by agarose and polyacrylamide gel electrophoreses, and by Southern blotting followed by hybridization to ^{32}P-labeled Ad12 DNA that fragments smaller than the 409-bp *Pst*I R fragment were not generated by the cleavage of Ad12 DNA with *Pst*I (data not shown). The polyacrylamide gels used in this analysis resolved fragments as small as 50 bp. Thus *Pst*I fragments A to R represented the entire nucleotide sequence of Ad12 DNA. In Fig. 1, the *Pst*I map of Ad12 DNA, as derived from the nucleotide sequence, is presented with all the primers used in the sequence determination of both DNA complements.

In the functional analysis of the nucleotide sequence of Ad12 DNA the following software programs were used. Data bases were searched with the programs blastn (1), tblastn (1),

* Corresponding author. Mailing address: Institute of Genetics, University of Cologne, Weyertal 121, D-50931 Cologne, Germany. Phone: 49-221-470-2386. Fax: 49-221-470-5163.

† Present address: Diagen, Hilden, Germany.

TABLE 1. Nucleotide sequences of Ad12 DNA

EMBL data bank identifier	Nucleotide nos.	Location in the Ad12 genome	Reference(s)
X73487	1–34125	Complete genome	This report
AD1201	1–3957	Left end	3
ADXH3G	1–2320	*Hin*dIII G fragment	41
AD12E1A	1–530	E1A gene	37
ADXTR1	8–202	Left ITR	38, 46
ADLEIBH12	1488–3861	E1B region	3, 26
ADLE2B	4831–10470	E2B region	39
ADRMUVI	16548–17705	Virion precursors pMu and pVI	21
AD12PROT	20400–21203	Endoprotease gene	25
AD1203	20966–22901	Parts of the 23- and 100-kDa proteins	27
ADERE4	30624–34117	Early region E4	24
AD12ITR	33758–34124	Left ITR transferred to the right end	36
ADXTR2	33980–34118	Right ITR	38, 46

blastp (1), fasta (31), or tfasta (31). The overall alignment of the nucleotide sequences of Ad2 and Ad12 DNA was performed with the program clustalV (23). For the construction of maps and patterns, other published procedures were used (10). The following data bases were explored: EMBL DNA release 33 and updates (40), SwissProt Protein release 23 (2), TFD Transcription Factor Database release 6 (22), and EPD Eucaryotic Promoter Database release 33 (5). The amino acid translation of DNA, performed with the program MAP, was presented in one single file in which open reading frames (ORFs) and the DNA sequence were merged. The program pepex was written here. This program allowed us to derive the peptide sequence with a specified size range and to write it into a separate sequence file in an appropriate data format. This program and the tools necessary to extract the needed information from the different data bases were realized in the programming language PERL, which was suited (49) to such purposes. The hardware used was a VAX model 3200, operating system VMS 5.3, and a Decstation model DECst 5000/125, operating system Ultrix 4.2, both from Digital Equipment Co.

Computer analyses of the reported Ad12 DNA nucleotide sequence also ascertained that this sequence was not contaminated with nucleotide sequences derived from the pBluescript KS, pBR322, or λ DNA vectors. Human cellular DNA sequences known from the SYREC2 DNA molecule (8) were not detected in the Ad12 nucleotide sequence either.

Nucleotide sequence accession number. The nucleotide sequence of 34,125 bp of the Ad12 genome has been communicated to the EMBL data library under EMBO accession number X73487.

RESULTS

Molecular weight and base, dinucleotide, and trinucleotide compositions of Ad12 DNA. Table 2 compiles the base, dinucleotide, and trinucleotide compositions of Ad12 DNA. The molecular weight calculated from the nucleotide composition of the Ad12 DNA molecule amounts to 22×10^6 and compares with values of 19.5×10^6 and 22.1×10^6 as determined previously by velocity sedimentation at alkaline pH and by length measurements with the electron microscope of the Ad12 DNA molecule, respectively (17).

The DNA molecule of Ad2 is 1,812 nucleotide pairs longer than Ad12 DNA (Tables 2 and 3). A comparison of dinucleotide and trinucleotide frequencies between Ad2 and Ad12 DNA reveals that the 5′-CG-3′ dinucleotide is underrepresented in Ad12 DNA, like in cellular DNA but unlike in Ad2 DNA, whereas 5′-TA-3′ and 5′-AT-3′ are low in Ad2 DNA. There are striking overrepresentations of AAA and TTT in Ad12 DNA (Table 2).

ITRs in Ad12 DNA. Inverted terminal repeats (ITRs) are characteristic of adenovirus DNAs. The ITR of Ad12 DNA is a perfect match of 161 nucleotide pairs (Fig. 2a), as previously reported (46). It is striking that beyond the perfect ITR, there are regions between nucleotides 162 and 237 on the left and nucleotides 33873 and 33948 of the opposite strand on the right end of the Ad12 molecule which exhibit about 64% homology (Fig. 2, bottom). In Ad2 DNA, the ITR extends over 102 nucleotides, and beyond that sequence only occasional homologies exist (Fig. 2b). Nucleotide sequence comparisons between the left (Fig. 3a) and right (Fig. 3b) terminal 500

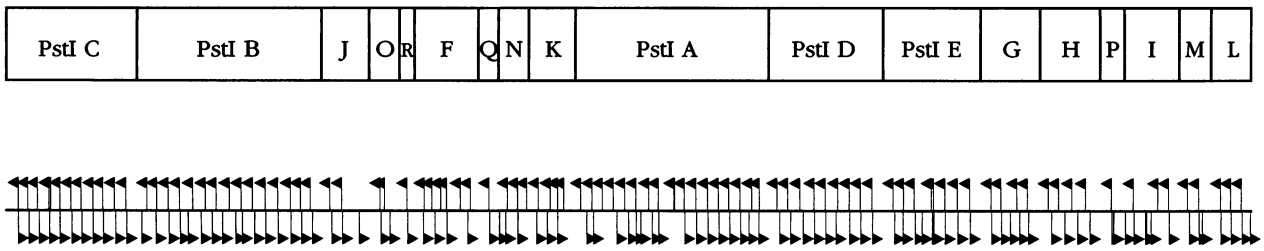

FIG. 1. *Pst*I restriction map of Ad12 DNA. The locations of all the primers used in the determination of the nucleotide sequence of the rightward-transcribed strands (arrows pointing to the left) and of the leftward-transcribed strands (arrows pointing to the right) are indicated. As a safeguard against possible sequencing errors, the nucleotide sequences of both strands were determined.

TABLE 2. Base, dinucleotide, and trinucleotide compositions of double-stranded Ad12 DNA[a]

Nucleotide	% Composition	Nucleotide	% Composition	Nucleotide	% Composition	Nucleotide	% Composition
A	26.74	C	23.26	G	23.26	T	26.74
AA	8.72	AC	5.79	AG	6.19	AT	6.04
CA	6.95	CC	5.73	CG	4.40	CT	6.19
GA	5.27	GC	6.47	GG	5.73	GT	5.79
TA	5.81	TC	5.27	TG	6.95	TT	8.72
AAA	3.12	AAC	1.82	AAG	1.90	AAT	1.88
ACA	1.85	ACC	1.42	ACG	0.97	ACT	1.55
AGA	1.43	AGC	1.71	AGG	1.50	AGT	1.55
ATA	1.28	ATC	1.16	ATG	1.72	ATT	1.88
CAA	1.98	CAC	1.40	CAG	1.84	CAT	1.72
CCA	1.88	CCC	1.32	CCG	1.03	CCT	1.50
CGA	0.86	CGC	1.54	CGG	1.03	CGT	0.97
CTA	1.18	CTC	1.26	CTG	1.84	CTT	1.90
GAA	1.72	GAC	1.12	GAG	1.26	GAT	1.16
GCA	1.76	GCC	1.47	GCG	1.54	GCT	1.71
GGA	1.52	GGC	1.47	GGG	1.32	GGT	1.42
GTA	1.45	GTC	1.12	GTG	1.40	GTT	1.82
TAA	1.89	TAC	1.45	TAG	1.18	TAT	1.28
TCA	1.46	TCC	1.52	TCG	0.86	TCT	1.43
TGA	1.46	TGC	1.76	TGG	1.88	TGT	1.85
TTA	1.89	TTC	1.72	TTG	1.98	TTT	3.12

[a] The analyses cover 34,125 nucleotides.

nucleotides of Ad2 and Ad12 DNA reveal numerous stretches of short nucleotide sequence homologies.

A similarity score between the entire nucleotide sequences of Ad2 and Ad12 DNAs is presented in Fig. 4. For this comparison a window of 500 nucleotides was used and shifted by one position along the alignment. There are many regions of greater than 70% homology between the two DNA sequences. Of course, such an overall alignment must be interpreted with caution. Since this alignment reflects relative positional information in the two adenovirus genomes, including gaps in the alignment, it is not possible to superimpose this alignment map on the functional map of Ad12 DNA (see Fig. 7). Nevertheless, the region of high homology between Ad2 and Ad12 DNAs in alignment positions 5000 to 8000 (Fig. 4) corresponds to the coding regions of the Ad2 and Ad12 DNA polymerases. The similarity between the two viral nucleotide sequences falls off in the extensively spliced E3 and E4 regions in the right halves of the genomes. The low similarity value around position 11000 is due to a computational alignment algorithm and to the fact that about 250 nucleotide pairs in this region of Ad2 DNA are lacking in Ad12 DNA.

Parts of the Ad12 DNA sequence were determined previ-

TABLE 3. Base, dinucleotide, and trinucleotide composition of double-stranded Ad2 DNA[a]

Nucleotide	% Composition	Nucleotide	% Composition	Nucleotide	% Composition	Nucleotide	% Composition
A	22.40	C	27.60	G	27.60	T	22.40
AA	6.08	AC	5.63	AG	6.20	AT	4.49
CA	6.92	CC	7.72	CG	6.76	CT	6.20
GA	5.42	GC	8.82	GG	7.72	GT	5.63
TA	3.97	TC	5.42	TG	6.92	TT	6.08
AAA	2.01	AAC	1.46	AAG	1.58	AAT	1.03
ACA	1.45	ACC	1.81	ACG	1.22	ACT	1.15
AGA	1.34	AGC	1.87	AGG	1.84	AGT	1.15
ATA	0.90	ATC	1.08	ATG	1.48	ATT	1.03
CAA	1.66	CAC	1.70	CAG	2.08	CAT	1.48
CCA	2.05	CCC	1.95	CCG	1.89	CCT	1.84
CGA	1.04	CGC	2.61	CGG	1.89	CGT	1.22
CTA	0.91	CTC	1.63	CTG	2.08	CTT	1.58
GAA	1.34	GAC	1.38	GAG	1.63	GAT	1.08
GCA	2.04	GCC	2.31	GCG	2.61	GCT	1.87
GGA	1.66	GGC	2.31	GGG	1.95	GGT	1.81
GTA	1.09	GTC	1.38	GTG	1.70	GTT	1.46
TAA	1.07	TAC	1.09	TAG	0.91	TAT	0.90
TCA	1.38	TCC	1.66	TCG	1.04	TCT	1.34
TGA	1.38	TGC	2.04	TGG	2.05	TGT	1.45
TTA	1.07	TTC	1.34	TTG	1.66	TTT	2.01

[a] The analyses cover 35,937 nucleotides.

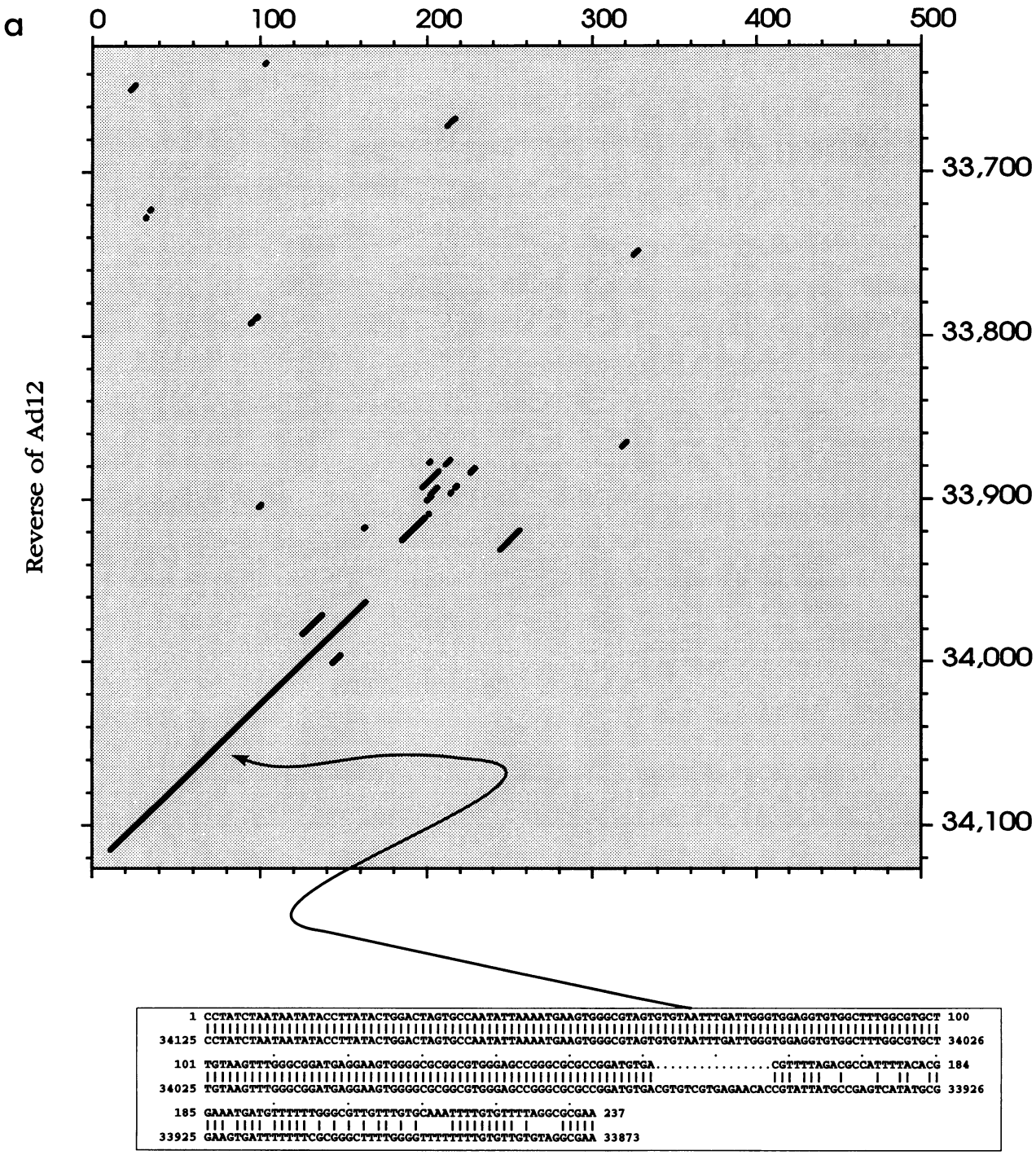

FIG. 2. Nucleotide sequence comparisons of the ITR of Ad12 DNA (a) and Ad2 DNA (b). The program Compare was used, the window was set at 21, and the stringency was set at 14.0.

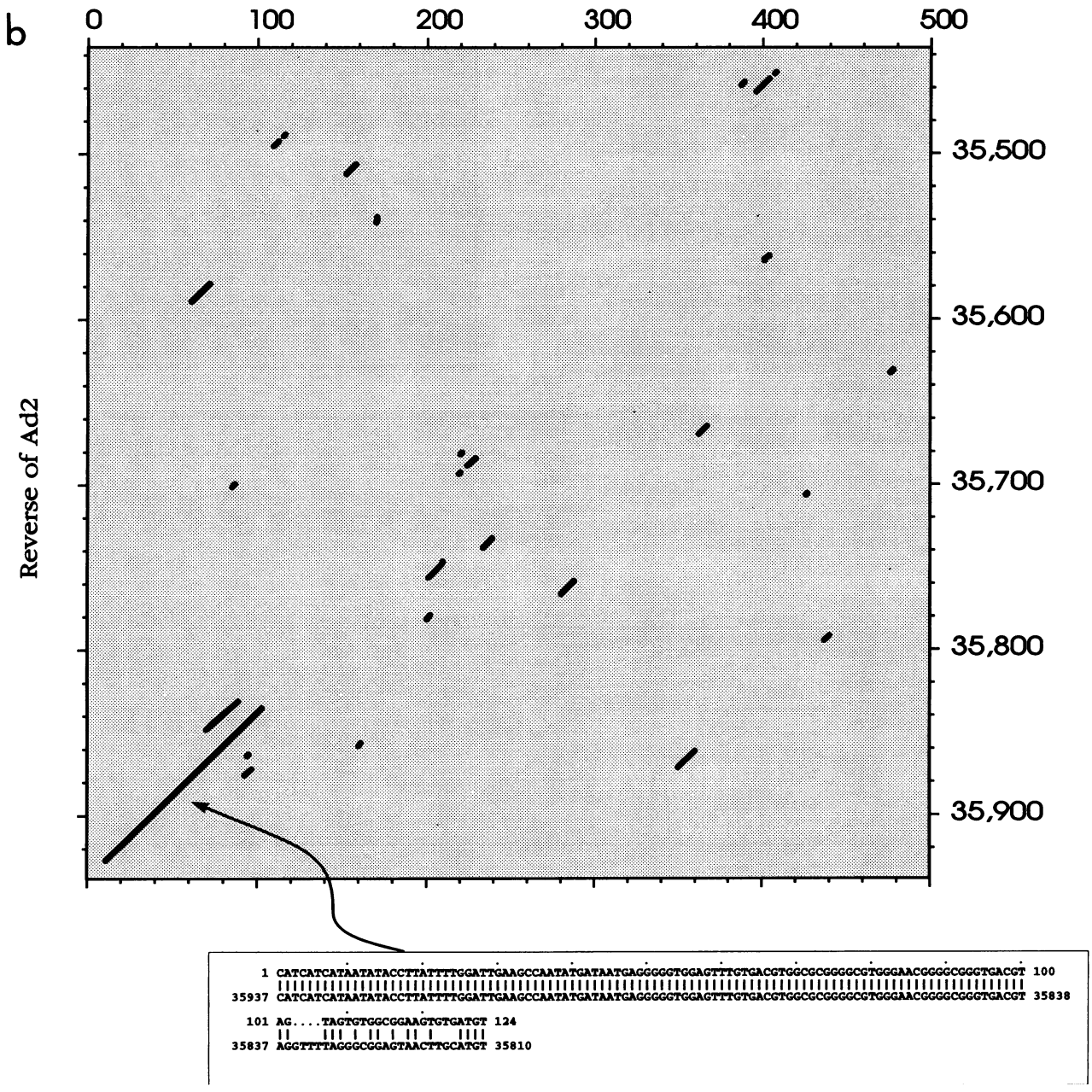

FIG. 2—*Continued*

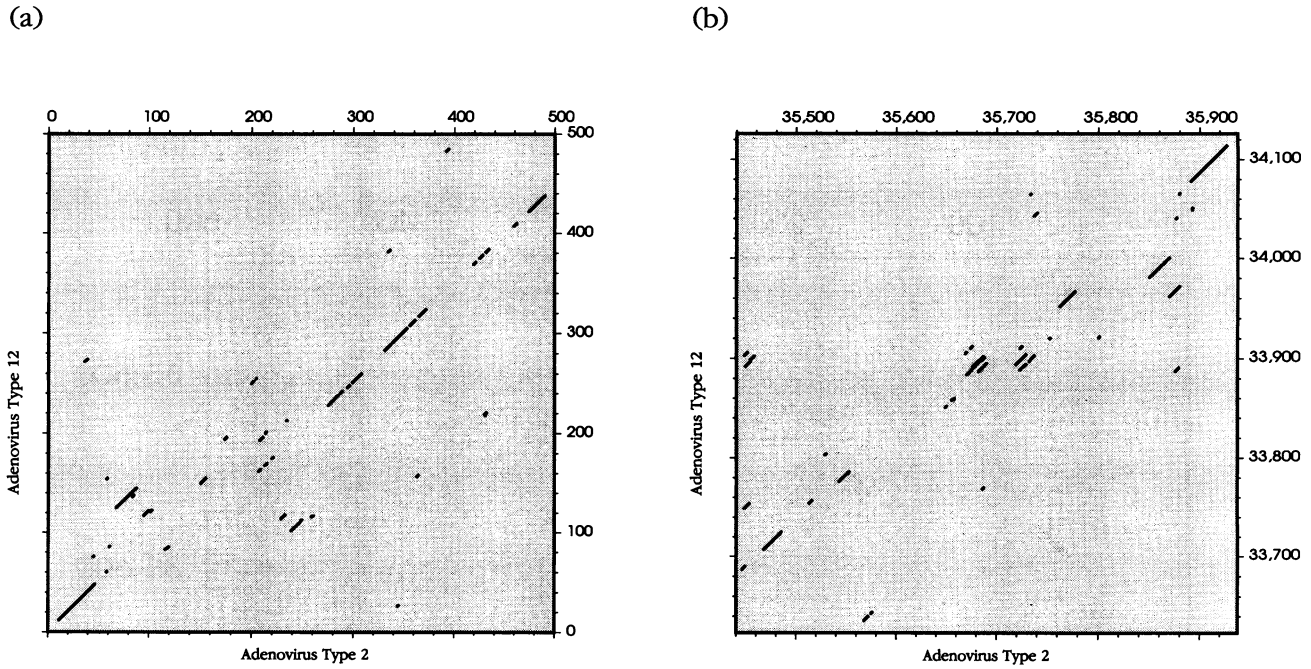

FIG. 3. Comparisons of the ITRs of Ad12 and Ad2 DNAs for the left (a) and right (b) termini. Comparisons were performed as described in the legend to Fig. 2.

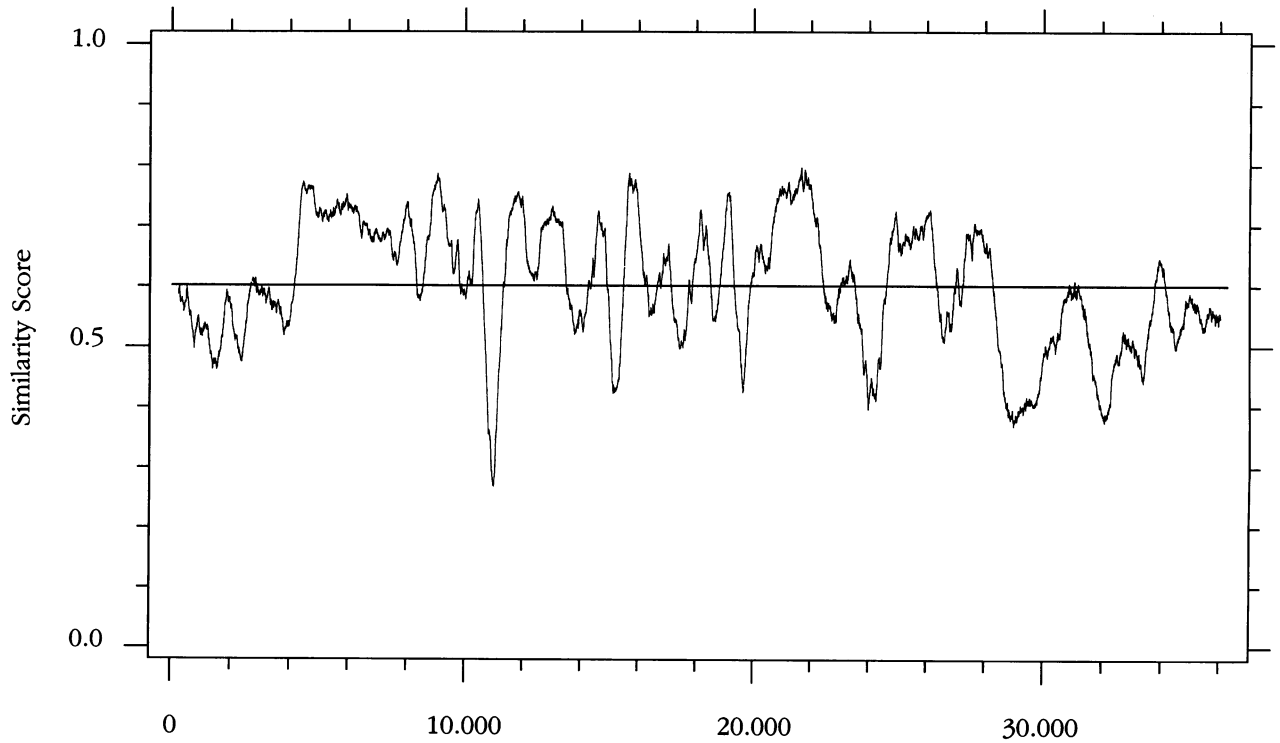

FIG. 4. Overall similarity scores of the entire nucleotide sequences of Ad12 and Ad2 DNAs. The program Clusta V was used to construct these scores. The numbers on the abscissa indicate positions in the alignment between Ad2 and Ad12 DNAs, not sequence numbers, since the two nucleotide sequences differ in length.

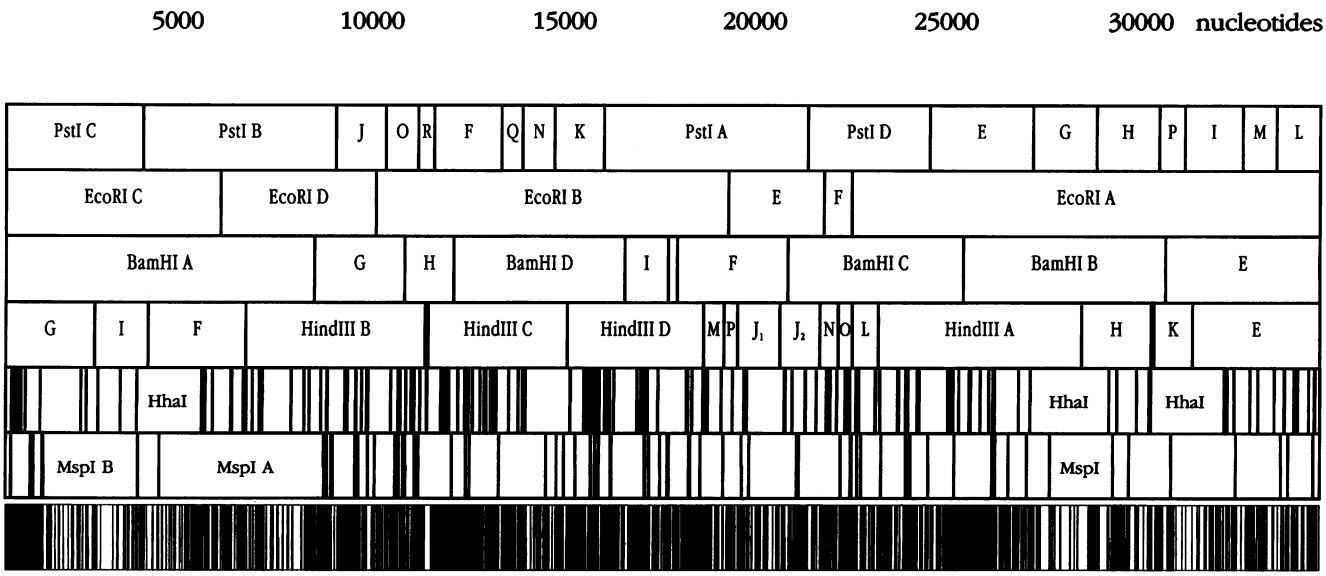

FIG. 5. Restriction maps of Ad12 DNA. From top to bottom the *Pst*I, *Eco*RI, *Bam*HI, *Hin*dIII, *Hha*I, and *Msp*I restriction maps of Ad12 DNA, derived from the primary nucleotide sequence, are shown. The bottom-most row presents all 1,500 5′-CG-3′ sequences in Ad12 DNA. The program MAPPLOT was used for the construction of these maps. The length scale in nucleotides is shown above the figure.

ously (for a review, see reference 48). At that time, different laboratories reported some variation in the sequence of the eight left terminal nucleotides. This variation might reflect different origins of the Ad12 inocula used in different laboratories and might, at the same time, be a consequence of the ability of Ad12 DNA to recombine with host DNA (9). The Ad12 DNA, which we obtained from the American Type Culture Collection and passaged only once, had the 5′-terminal sequence 5′-CCTATCTAATAATATA . . . (Fig. 2), which was also one of the results in the earlier comparison (48).

Restriction maps for the Ad12 genome. In Fig. 5 selected restriction maps of the Ad12 genome are presented. Some restriction maps of Ad12 DNA have been published elsewhere (45). At the time of this writing, restriction enzymes that did not cleave Ad12 DNA were not known. It was of interest that the restriction endonuclease *Bst*EII from *Bacillus stearothermophilus*, with the recognition sequence 5′-GGTNACC-3′ (28), could cut Ad12 DNA only once at nucleotide 3444. Thus, about 90% of the Ad12 genome can be cloned as one contiguous fragment.

Since one of our motivations to determine the nucleotide sequence of Ad12 DNA has been to facilitate more detailed studies of the patterns of de novo methylation in integrated Ad12 DNA, a presumptive defense mechanism of the host (14), it is worth mentioning that the presence of 75 5′-CCGG-3′ (*Hpa*II-*Msp*I) and of 181 5′-GCGC-3′ (*Hha*I) recognition sites (*Msp*I and *Hha*I maps are shown in Fig. 5) contrasts with the presence of 1,500 5′-CG-3′ sequences (bottom row in Fig. 5), which are the preferred target sites for the host cell DNA methyltransferase system. Thus, by analyzing the methylation status of *Hpa*II and *Hha*I recognition sequences, as is frequently done in work on DNA methylation, one would score only about 17% of the sequences that could become methylated in the integrated Ad12 DNA. This obvious discrepancy constitutes a problem for much of the work on DNA methylation and can be overcome only by applying the genomic sequencing method (7, 32, 34, 47). This method permits the determination of 5′-methyldeoxycytidine residues at all 5′-CG-3′ sequences. A graphic evaluation of the 5′-CG-3′, 5′-GCGC-3′, and 5′-CCGG-3′ sequences in the Ad2 (Fig. 6a) and Ad12 (Fig. 6b) DNAs is presented in Fig. 6. The patterns of distribution show gross similarities but differ in many details.

ORFs, promoters, TATA sequence, and polyadenylation signals in the Ad12 genome. In Fig. 7, the ORFs detected in

TABLE 4. Mapping of known promoter sequences and comparison with the Ad12 nucleotide sequence presented here

EPD no.[a]	Adenovirus type	Name	Estimated % similarity	Nucleotide no.	
				Start[b]	End[b]
EPD30061	Ad12	E1A P1	99	9	407
EPD11197	Ad12	E1a P2+	99	9	546
EPD07149	Ad2	E1a	63	47	480
EPD11195	Ad5	E1a	62	47	480
EPD11196	Ad7	E1a	71	153	480
EPD17110	Sa7p	E1a	65	8	476
EPD07151	Ad7	E1b	56	995	1326
EPD07152	Ad12	E1b	100	1029	1628
EPD26036	Ad12	MLP[c]	99	5303	5902
EPD07159	Ad2	MLP	74	5303	5902
EPD11203	Ad5	MLP	74	5303	5902
EPD11204	Ad7	MLP	73	5303	5899
EPD11202	Ad5	IX	60	2878	3464
EPD07158	Ad7	IX	61	2877	3469
EPD07157	Ad2	IX	59	2877	3467
EPD07154	Ad2	E3	72	25548	25963
EPD11199	Ad5	E3	76	25770	25963
EPD11200	Ad5	IVa2	74 (rev)[d]	6089	5491
EPD07156	Ad2	IVa2	74 (rev)	6089	5491
EPD11201	Ad7	IVa2	73 (rev)	6089	5491
EPD07153	Ad2	E2a	75 (rev)	25963	25423
EPD07160	Ad2	E2 late	74 (rev)	24943	24472

[a] These identifiers present the nomenclature of the Eucaryotic Promoter Database (EPD).
[b] Nucleotide numbers in the Ad12 DNA sequence determined in this study.
[c] MLP, major late promoter.
[d] Sequence on leftward-transcribed DNA strand.

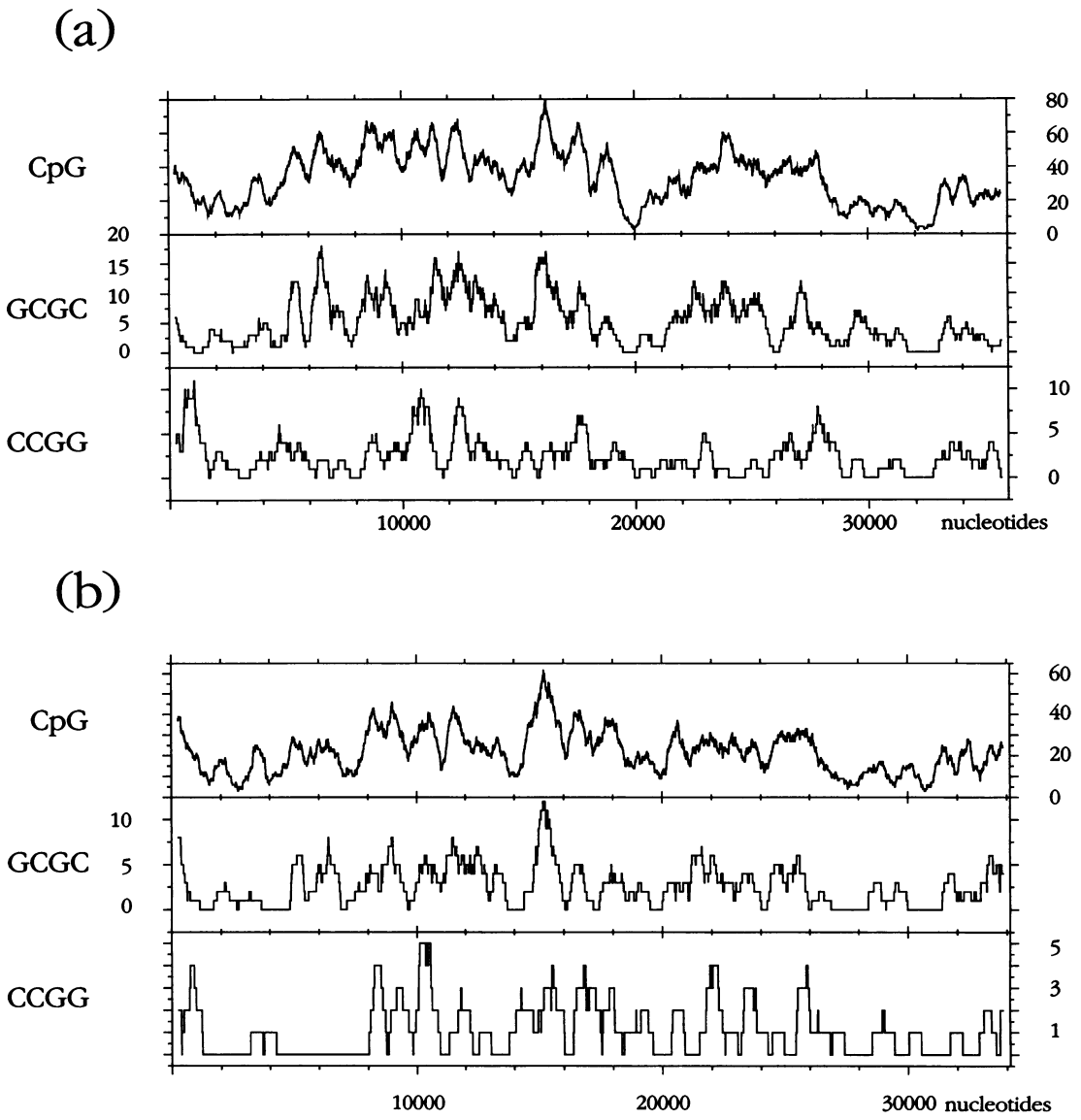

FIG. 6. Distribution of the CpG, GCGC, and CCGG sequences across the Ad2 (a) and Ad12 (b) DNA molecules. The program Window was used to generate these patterns.

the Ad12 nucleotide sequence in all three reading frames and on both complements of Ad12 DNA are presented. The ORFs were derived from the nucleotide sequence and have not yet been independently confirmed. This figure also shows some of the Ad12 promoters, the TATA sequence, and polyadenylation signals. The symbols used in this composite map are explained in the legend to Fig. 7. ORFs shorter than 80 nucleotides are not considered significant and have not been included in Fig. 7.

In Table 4, the nucleotide sequences in Ad12 DNA with similarities or near identities to known and published sequences from other adenovirus promoters were listed. Although it is very likely that the Ad12 DNA sequences correspond to the named genes of other adenoviruses, this possibility has not been confirmed by functional tests in most instances. The nomenclature for the Ad12 ORFs should therefore be regarded as preliminary and must await functional confirmation.

We refrained from sequence comparisons of splicing signals, since such assignments on the Ad12 DNA sequence would be misleading if based solely on nucleotide sequence analyses. With a few exceptions, such comparisons could not yet be backed up by mapping data for Ad12-specific mRNAs on the Ad12 genome as obtained from biochemical experiments.

DISCUSSION

We have determined the sequence of the 34,125 nucleotide pairs in the Ad12 DNA molecule and have compared this sequence with the structures of other adenovirus genomes, in particular that of Ad2 DNA (4). In many aspects, the distribution of viral functions on the Ad12 genome reflects that in Ad2 DNA with minor differences. The functional map of Ad12 DNA is in agreement with the mapping of the early Ad12

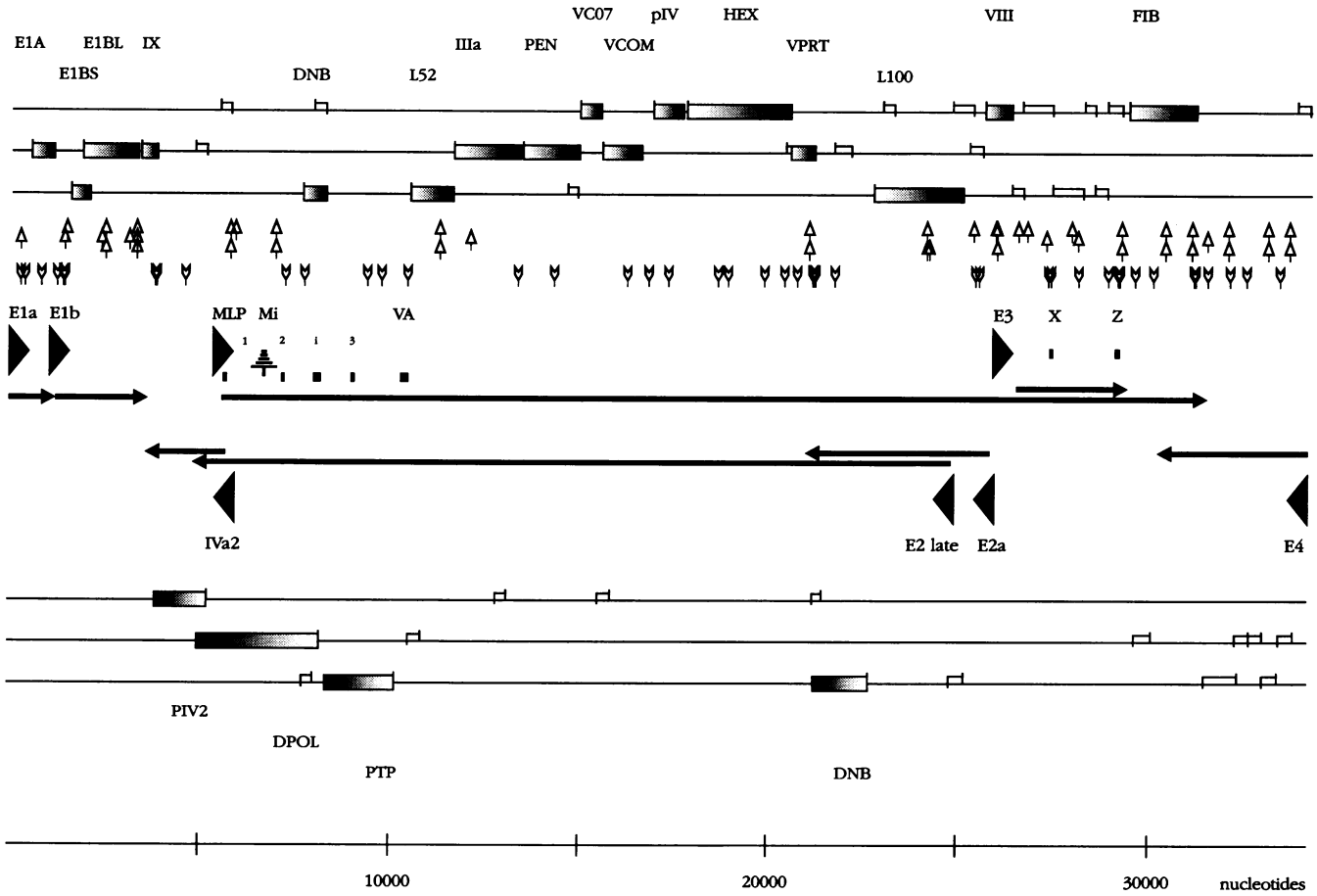

FIG. 7. Functional map of the Ad12 genome. ORFs in all three registers for the rightward-transcribed (top) and leftward-transcribed (bottom) strands are indicated. The nomenclature follows that customary for Ad2 DNA. The map details the ORFs in the E1A, E1B, E2A, E2B, E3, and E4 regions. Some of the reading frames, particularly in the E3 and E4 regions, have not been experimentally determined. The major late promoter (MLP), the Ad12 mitigator (Mi) (50, 51), the gene for the virus-associated (VA) RNA, and the leaders 1, 2, i, 3, x, and z have also been designated. Other abbreviations include DBP (DNA-binding protein), Ptp (precursor terminal protein), DPOL (Ad12-specific DNA polymerase), HEX (hexon protein), PEN (penton), FIB (fiber protein), VPRT (viral protease), VC07 (major core precursor of protein VII), and VCOM (minor core protein, protein V). The ORFs for the viral proteins IX, IV, IIIa, L52 (L1 region), and L100 are also shown. TATAAA, TATAAT, and TATAWAW sequences are represented by open arrowheads, and AATAA polyadenylation signals are represented by arrow tails. Below the figure a scale in nucleotides serves as a frame of reference.

functions on the genome by previously published DNA-RNA hybridization and cell-free translation experiments (19, 20, 30).

The ORF in Ad12 DNA homologous to the virus-encoded endoprotease of Ad2 DNA (25) is preceded by a 5.8-kbp unidentified reading frame that lies adjacent to the virus-encoded protease in the same reading frame. We have resequenced this DNA segment several times by using different DNA polymerases, but we have unequivocally come up with the same result.

The availability of the entire nucleotide sequence of Ad12 DNA will facilitate more detailed studies of this virus, which represents one of the strongest oncogenic agents for rodents among the human adenoviruses. For our own work on the mechanisms of DNA integration and the de novo methylation of integrated Ad12 genomes, this information will be extremely useful, since it will permit further work on details of the transcriptional map.

The entire nucleotide sequence of Ad12 DNA has already been made generally available. A detailed annotation of the nucleotide sequence can be found there and will not be repeated here.

ACKNOWLEDGMENTS

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