MAPPING OF THE *ECO*RI B FRAGMENT-SPECIFIC EARLY mRNA SPECIES OF ADENOVIRUS TYPE 2

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1. Introduction

The early genes of the adenovirus type 2 genome (M_r 23 × 10^6) are clustered in four separate gene blocks. These are transcribed to the right at the approximate map position 1–11 and 75–86 and to the left at the coordinates 66–72 and 93–99 [1,2]. Five major early messenger RNA species with app. mol. wt 1.4 × 10^6, 9.4 × 10^5, 8.4 × 10^5, 8.4 × 10^5 and 4.2 × 10^5 have been isolated from Ad 2-infected KB cells [3]. The approximate map positions of these mRNA species have been determined by their homology to viral DNA restriction fragments. For most early gene regions useful restriction fragments have been available for analysis. For the second early gene block, represented by the *Eco*RI B fragment, however, cleavage maps have been established only recently. In this paper the position of the early mRNA species (M_r 8.4 × 10^5) which is the leftward transcript of the *Eco*RI B fragment (map position 58.5–70.7) has been determined by in situ hybridisation of labelled RNA [4] to subfragments of the *Eco*RI B fragment produced by the restriction endonucleases *Bgl* II and *Hin*cII. Averaging the results from three experiments with *Bgl* II and *Hin*cII, respectively, we calculated the following coordinates for this mRNA: 61.1 ± 0.4 for the 3′-terminus and 68.4 ± 0.4 for the 5′-terminus. The coordinates obtained for the mRNA by this approach agree with electron microscopic data [5,6]. The method used here for positioning is straightforward and hopefully also applicable for approximate orientation of other mRNAs.

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2. Materials and methods

2.1. *Isolation of polysomal RNA*

Polysomes were isolated from [^{3}H]uridine-labelled KB cells 6 h after infection with adenovirus type 2 (Ad 2) and early polysomal RNA was prepared as in [7].

2.2. *Restriction endonucleases*

The restriction endonucleases *Bgl* II [8] and *Hin*cII [9] were isolated by ammonium sulfate fractionation and by phosphocellulose and DEAE-cellulose chromatography. The preparation of the enzymes *Eco*RI and *Sma*I have been described in [3].

2.3. *Restriction fragments*

The conditions for digestion of viral DNA with restriction enzymes, the resolution of fragments in 1.4% agarose gels and the isolation of the *Eco*RI B fragment of Ad 2 has been detailed in [3].

2.4. *Hybridisation of early RNA to the* Eco*RI B subfragments*

The *Bgl* II and *Hin*cII subfragments of *Eco*RI B were resolved on 1.4% agarose gels and transferred to cellulose nitrate sheets as in [4]. Hybridisation of the labelled early polysomal RNA was carried out in 0.9 M NaCl, 0.09 M Na-citrate (6 × SCC), 1% sodium dodecylsulfate (pH 7.4) at 68°C. To remove non-hybridised RNA the filters were RNase treated [10]. For detection of hybridised RNA the transferred fragments were fluorographed and scanned at 540 nm [11,12]. In parallel the radioactive bands of hybridised RNA were cut out from the cellulose nitrate sheets and counted in a toluene based scintillator.

3. Results and discussion

3.1. *Coordinates and molecular weights of the* Bgl *II and* Hinc*II subfragments of* Eco*RI B*

Based on the *Bgl* II cleavage map of the *Eco*RI B fragment (M. Zabeau, R. Roberts, personal communication) we deduced the order of the three *Hin*cII subfragments of *Eco*RI B by reciprocal digestion of the fragments. Since we did not know the order of the *Hin*cII fragments of the total Ad 2 genome we designated the fragments in descending size *Eco*RI B/*Hin*cII-1, *Eco*RI B/*Hin*cII-2, and *Eco*RI B/*Hin*cII-3. The molecular weights of the *Bgl* II and *Hin*cII subfragments of the *Eco*RI B fragment were determined by relating their electrophoretic mobility (fig.1) to that of *Eco*RI B [13] and *Sma*I (C. Mulder, personal communication) marker fragments of Ad 2. The values obtained are given in table 1. The coordinates

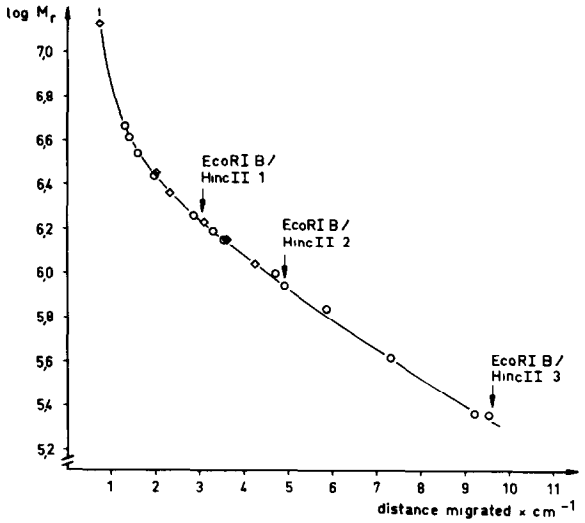

Fig.1. Determination of the molecular weights of the *Hin*cII subfragments of *Eco*RI B. Plot of the distance migrated of the marker fragments: *Eco*RI fragments of Ad 2 (mol. wt ($\times 10^{-6}$): A, 13.55; B, 2.8; C, 2.33; D, 1.70; E, 1.40; F, 1.13) and *Sma*I fragments of Ad 2 (mol. wt $\times 10^{-6}$): A, 4.58; B, 4.05; C, 3.44; D, 2.97; E, 1.83; F, 1.55; G, 1.42; H, 1.01; I, 0.87; J, 0.69; K, 0.41; L, 0.23; M, 0.23) versus the logarithm of their molecular weights. The arrows denote the distance migrated of the *Hin*cII subfragments of *Eco*RI B in the same gel. The molecular weights of the *Bgl* II subfragments of *Eco*RI B were determined in the same fashion (◇——◇) *Eco*RI fragments of Ad 2; (○——○) *Sma*I fragments of Ad 2.

Table 1
Molecular weights of the *Bgl* II and *Hin*cII subfragments of *Eco*RI B

Name of subfragment	Mol. wt $\times 10^{-6}$
*Eco*RI B/*Bgl* II C	1.55
Bgl II J	0.83
*Eco*RI B/*Bgl* II D	0.37
*Eco*RI B/*Hin*cII-1	1.70
*Eco*RI B/*Hin*cII-2	0.89
*Eco*RI B/*Hin*cII-3	0.20

of the *Eco*RI B fragments are shown in table 3 and fig.4.

3.2. *Extent of transfer of the* Eco*RI B subfragments to cellulose nitrate sheets*

To determine the extent of transfer of the denatured DNA to the cellulose nitrate sheets ^{32}P-labelled DNA was prepared. Two litres of Ad 2-infected KB cells were labelled from 18–42 h post infection with 5 μCi/ml [^{32}P]orthophosphoric acid (NEX-054). The virus was purified, the labelled DNA (spec. act. 9.76×10^4 cpm/μg) was extracted, digested with the *Eco*RI endonuclease and the *Eco*RI B fragment was isolated after resolution of the fragments on 1.4% agarose gels. The *Eco*RI B fragment was digested with the enzymes *Hin*cII and *Bgl* II and 3 μg aliquots of the subfragments were resolved on 1.4% agarose gels and transferred to cellulose nitrate sheets. The fragments were localised by ethidium bromide fluorescence and autoradiography, cut out and counted in a scintillation counter. 52.6% of the counts of the *Hin*cII subfragments and 59.8% of the counts of the *Bgl* II subfragments layered on the gel were found in the transferred fragment bands (table 2). The two smallest fragments are obviously under-represented, they were not considered since they are not homologous to the *Eco*RI B-specific mRNA species.

3.3. *Hybridisation of early mRNA to the* Bgl *II and* Hinc*II subfragments of* Eco*RI B*

The molecular weight of the mRNA transcribed from the *Eco*RI B fragment is 8.4×10^5 as determined by electrophoresis in formamide polyacrylamide gels [3]. This mRNA is therefore homologous

Table 2
Transfer of ^{32}P-labelled *Eco*RI B subfragments to cellulose nitrate sheets

Name of subfragment	^{32}P cpm, (%) transferred[a]	
*Eco*RI B/*Bgl* II C	57 990	(38.7)
Bgl II J	24 977	(16.7)
*Eco*RI B/*Bgl* II D	6668	(4.4)
*Eco*RI B/*Hin*cII-1	55 580	(37.1)
*Eco*RI B/*Hin*cII-2	21 693	(14.5)
*Eco*RI B/*Hin*cII-3	1747	(1.2)

[a] Aliquots, 3 μg, of ^{32}P-labelled *Bgl* II and *Hin*cII digests of the *Eco*RI B fragment (on the day of analysis, spec. radioact., 50 000 ^{32}P cpm/μg) were electrophoresed in 1.4% agarose gels, transferred to cellulose nitrate sheets, cut out and counted in a toluene-based fluor

to 60% of the coding strand of the *Eco*RI B fragment corresponding to 7.3 map unit equiv. of the Ad 2 genome. In control experiments with increasing amounts of labelled RNA we verified that the transferred fragments were in excess during our hybridisation reactions. The percentage of radioactivity of the *Eco*RI B fragment-specific mRNA which stringently hybridised to a particular subfragment should therefore be proportional to the length of the mRNA fraction homologous to the subfragment. The *Eco*RI B fragment-specific mRNA hybridised to only two adjacent *Eco*RI B subfragments produced by *Bgl* II and *Hin*cII, respectively. Utilising the cleavage site between those two subfragments as landmark we could therefore position the termini of the mRNA by subtracting or adding the map unit equivalents of the hybridised RNA in both directions from the overlapped cleavage point. A representative experiment using *Bgl* II subfragments of *Eco*RI B is shown in fig.2 and a similar experiment with *Hin*cII subfragments is illustrated in fig.3. Using the *Bgl* II subfragments we obtained the coordinates 60.6 ± 0.4 and 67.9 ± 0.4 (av. 3 determinations) and taking *Hin*cII subfragments of *Eco*RI B we calculated the coordinates 61.5 ± 0.4 to 68.8 ± 0.4 for the *Eco*RI B fragment-specific mRNA as summarized in table 3. There was little difference between measuring the hybridised RNA by direct counting in scintillator or by fluorography. Comparing the results obtained by two sets of subfragments revealed that the results differed by less than one map unit (fig.4).

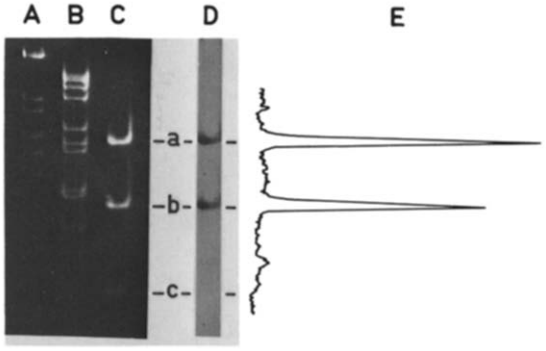

Fig.2. Hybridisation of early Ad 2 mRNA to the *Bgl* II subfragments of *Eco*RI B. In A and B the separation of the marker fragments are shown (A, *Eco*RI fragments of Ad 2; B, *Sma*I fragments of Ad 2) and in C the resolution of the *Bgl* II subfragments of *Eco*RI B (a, *Eco*RI B/*Bgl* II C; b, *Bgl* II J; c, *Eco*RI B/*Bgl* II D) in a 1.4% agarose gel stained with ethidium bromide and photographed. D represents a fluorogram and E the corresponding scan of the *Eco*RI B-specific mRNA hybridised to the subfragments *Eco*RI B/*Bgl* II C (a) and *Bgl* II J (b), but not to *Eco*RI B/*Bgl* II D (c). For hybridisation to 4 μg *Bgl* II-digested *Eco*RI B fragments we used between 2.2–3.35 × 10^6 ^{3}H cpm of early polysomal RNA (spec. act. 27 500–41 850 cpm/μg).

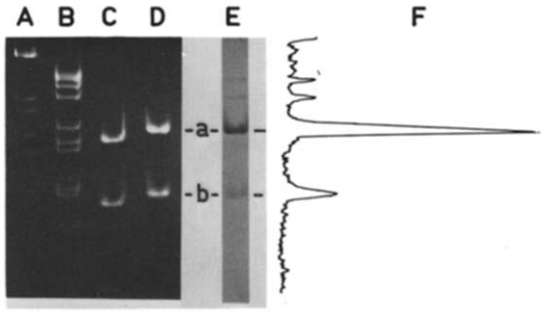

Fig.3. Hybridisation of early Ad 2 mRNA to the *Hin*cII subfragments of *Eco*RI B. A and B show marker fragments and C the *Bgl* II subfragments of *Eco*RI B as in the legend to fig.2. D depicts the *Hin*cII subfragments of *Eco*RI B (a, *Eco*RI B/*Hin*cII-1; b, *Eco*RI B/*Hin*cII-2; the very small fragment *Eco*RI B/*Hin*cII-3 is not visible in this photograph). E represents a fluorogram and F the corresponding scan of the *Eco*RI B-specific RNA hybridised to the subfragments *Eco*RI B/*Hin*cII-1 (a) and *Eco*RI B/*Hin*cII-2 (b). *Eco*RI B/*Hin*cII-3 does not hybridise. *Hin*cII-digested *Eco*RI B fragment, 4 μg, and the same RNA preparations as in fig.2 legend were used for hybridisation.

Table 3
Distribution of *Eco*RI B fragment-specific hybrids of early mRNA between subfragments of *Eco*RI B

Name of subfragment (map position)	Distribution of RNA hybridized between subfragments								Calculated coordinates of subfragment region homologous to mRNA[c]
	Calculated from fluorogram scan %			Map unit equiv.[a]	Calculated from radioactivity counted %, (^{3}H cpm)			Map unit equiv.[a]	
	1	2	3		1	2	3		
*Eco*RI B/*Bgl* II C (63.9[b] to 70.7)	55	52	54	3.9	51 (831)	59 (5104)	53 (1760)	4.0	63.9[b] to 67.9 (63.9[b] + 3.95)
Bgl II J (60.2 to 63.9[b])	45	48	46	3.4	49 (808)	41 (3522)	47 (1573)	3.3	63.9[b] to 60.6 (63.9[b] − 3.35)
*Eco*RI B/*Bgl* II D (58.5 to 60.2)	0	0	0	0	0 (0)	0 (0)	0 (0)	0	–
*Eco*RI B/*Hinc*II-1 (63.3[b] to 70.7)	77	77.5	81	5.7	72 (788)	75 (6168)	73 (2077)	5.4	63.3[b] to 68.8 (63.3[b] + 5.55)
*Eco*RI B/*Hinc*II-2 (59.4 to 63.3[b])	23	22.5	19	1.6	28 (306)	25 (2052)	27 (754)	1.9	63.3[b] to 61.5 (63.3[b] − 1.75)
*Eco*RI B/*Hinc*II-3 (58.5 to 59.4)	0	0	0	0	0 (0)	0 (0)	0 (0)	0	–

[a] The total *Eco*RI B-specific mRNA (M_r 7.8 × 10^5) corresponds to 7.3 map unit equiv. of the Ad 2 genome. Numbers are averaged from 3 experiments
[b] Cleavage point which is overlapped by the *Eco*RI B transcript
[c] Map unit equivalents were averaged and added to (fragments to the right) and subtracted from (fragments to the left) of the overlapped cleavage point coordinate

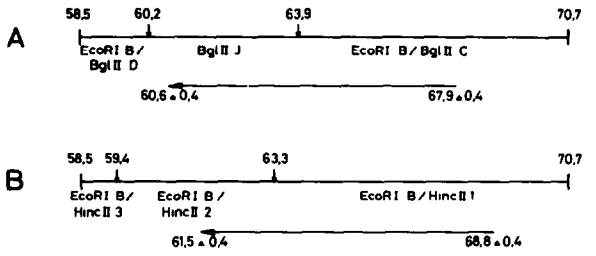

Fig.4. Localisation of the *Eco*RI B fragment-specific mRNA. Length and coordinates of the *Eco*RI B-specific mRNA in relationship to the *Bgl* II subfragments shown in A and to the *Hin*cII subfragments shown in B are represented by the horizontal arrow with its tip at the 3'-terminus of the mRNA.

The described procedure is reasonably accurate and relatively simple to conduct. It seems to be well suited for a first positioning of viral mRNAs.

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