THE BINDING OF Qβ INITIATOR FRAGMENTS TO *E. COLI* RIBOSOMES

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1. Introduction

The extensively studied bacteriophages of the Qβ, R17 and MS2 serotypes [1] each contain a single strand of RNA with three ribosome binding sites around which extended nucleotide sequences are now known [2–5, 7]. It is likely that the inter- and extracistronic regions preceding cistrons contain special oligonucleotide sequences or structures that enable ribosomes to select the AUG initiator triplet from among the many AUG-containing sequences [6]. The structural similarities that exist at different binding sites [13, 15] do not, however, readily account for this specificity, nor is there a clear idea of how much phage RNA is necessary for ribosome recognition and attachment.

It may be easier to determine which features of mRNA specify ribosome attachment by testing small fragments containing the initiator regions for their ability to rebind to ribosomes. Thus, Weber [7] showed that a 100 nucleotide fragment of Qβ RNA extending towards the 5′-end from the initiator AUG of the coat cistron bound well to ribosomes and concluded that the ribosome protected segment to the right of the AUG is not needed for the ribosome binding function. However, Adams et al. [4] were unable to detect any binding of a 59 nucleotide fragment from the corresponding region of R17 RNA. If Qβ RNA is degraded with alkali to fragments of average molecular weight less than 5 S, only the true initiation sites are recognised [8]. Moreover, 5′-terminal segments of Qβ RNA of approx. 170–300 nucleotides bind well to ribosomes [3, 9].

We have isolated fragments of Qβ RNA from the coat protein cistron ribosome binding site which range from 30–120 nucleotides in length [10] and show that only the longest of these form initiation complexes with *E. coli* ribosomes. The reasons for the failure of the shorter ones to rebind are discussed.

2. Materials and methods

Initiator fragments of [^{32}P]Qβ RNA were prepared by gel electrophoresis as previously described [10], except that 18 A_{260} units of autoradiolytically degraded [^{32}P]Qβ RNA (2.5×10^9 cpm) were bound to *E. coli* MRE 600 ribosomes in the presence of unfractionated fMet-tRNA$_F$ in the molar ratios 1:12:8, respectively. RNA was extracted from each major gel band by gentle homogenisation of the gel slice with water and 80–100 μg unlabelled yeast ribosomal RNA, precipitated in ethanol and dissolved in 0.035 ml RBS buffer (50 mM Tris-HCl pH 7.8, 50 mM NH_4Cl, 5 mM $Mg(CH_3COO)_2$ and 6 mM 2-mercaptoethanol).

Binding of full-length [^{14}C]Qβ RNA to *E. coli* ribosomes was carried out in parallel with that of the various Qβ initiator fragments. 0.16 A_{260} units [^{14}C]Qβ RNA were incubated with ribosomes and fMet-tRNA$_F$ in the molar ratios 1:12:8, respectively. The same quantities of ribosomes and fMet-tRNA$_F$ were incubated with fragments.

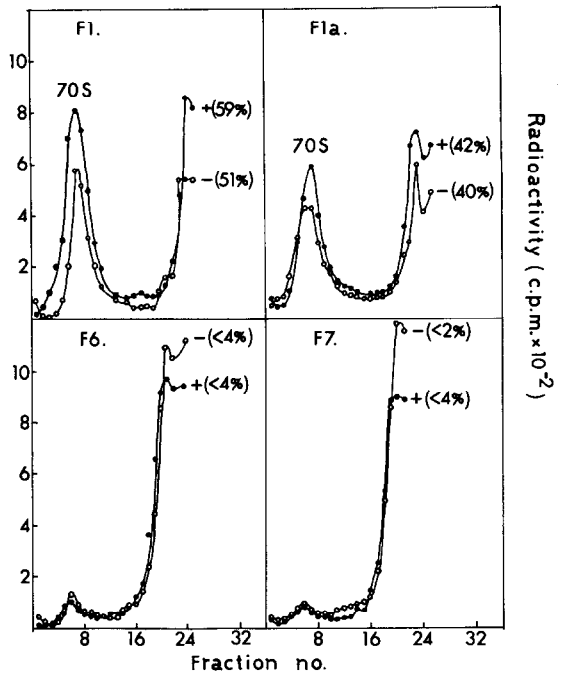

Fig. 1. Sucrose gradient analysis of 70 S complexes of *E. coli* MRE 600 ribosomes and ^{32}P-labelled Qβ initiator fragments (F). Each binding reaction was performed in 100 μl RBS buffer which contained: 1.6 A_{260} units fMet-tRNA$_F$, 0.3 mM GTP, 3.2 A_{260} units ribosomes and 3–5 × 10^4 ^{32}P cpm initiator fragment. The initiation complex was identified in the 70 S region of a 4–20% sucrose gradient [12]: (•—•—•) ^{32}P in a complete system, (o—o—o) ^{32}P in a control incubation to which fMet-tRNA$_F$ and GTP were not added. The percentage of each input fragment bound to ribosomes in the presence (+) and absence (−) of added fMet-tRNA$_F$ is indicated.

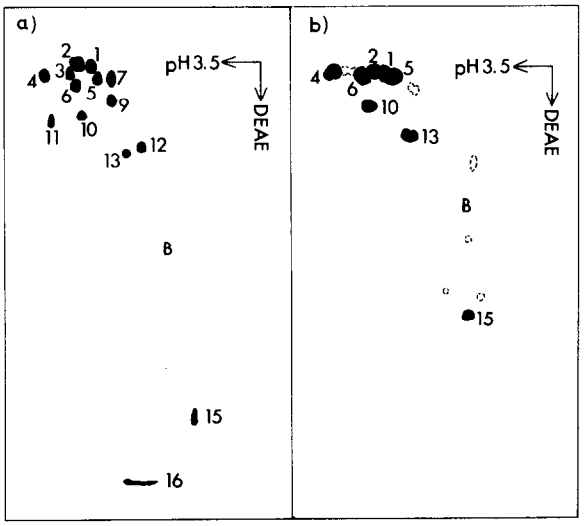

Fig. 2. T_1 RNAase fingerprints of Qβ initiator fragments. a) fragment 1a; b) fragment 5. Fig. 3 gives the location of each oligonucleotide. B marks the position of the blue marker (xylene cyanol).

3. Results

If a 70 S complex of Qβ RNA and ribosomes is incubated with T_1 or pancreatic RNAase at 22°C, short initiator regions in the mRNA (~ 25–35 nucleotides) are shielded from nuclease digestion and remain stably attached to the ribosomes [8, 12]. Preliminary experiments in our laboratory indicated that the isolated fragments did not rebind to fresh ribosomes under conditions in which 20% of intact [^{14}C]Qβ RNA bound. We were thus prompted to search for the shortest fragment that will form a 70 S complex.

Fig. 1 shows that extremely good binding of fragments 1 and 1a (fig. 3) was obtained both in the presence and absence of added fMet-tRNA$_F$ whereas 5 (not shown), 6 and 7 each gave less than 4% binding. The lack of fMet-tRNA$_F$ dependence may, however, imply some spurious binding. To investigate this possibility one third of the 70 S complex* of ribosomes and fragment 1 was incubated with pancreatic RNAase at 22°C for 10 min (enzyme to substrate ratio 1:40), then filtered through a Millipore filter (type HAWP 02500) and washed 4 times with chilled RBS buffer. The RNA adhering to the filter was isolated, digested at 37°C with T_1 RNAase, and subjected to one-dimensional electrophoresis on DEAE-cellulose paper [11]. All the spots were identified by further digestion with pancreatic RNAase [11, 17]. All were derived from the ribosome protected segment that overlaps the coat cistron, as described by Hindley and Staples [12]. A similar result was obtained with fragment 1a. This suggests that true initiation occurred, especially as both fragments contain only one AUG sequence. As the amount of each fragment present in

* 70 S complexes from the complete and control gradients (fig. 1) were separately treated with pancreatic RNAase.

the binding reaction was estimated to be < 0.1 μg, it is possible that sufficient endogenous ribosomal associated fMet-tRNA$_F$ was present to obviate the need for its addition.

The origin and chain length of fragments 1 and 1a were determined by isolating the RNA from each 70 S complex, digesting it with T_1 RNAase and separating the products by two-dimensional electrophoresis (fig. 2a) [11, 17]. The labelled oligonucleotides were identified from their position by comparison with T_1 maps obtained in previous experiments of authentic F1 and F1a. In addition, the T_1 fingerprint of F1a was the same both before and after rebinding. Fragments 5, 6 and 7 were identified as follows. The RNA not bound to ribosomes (fig. 1) was isolated and fingerprinted as for F1 and F1a, and the oligonucleotides identified from their pancreatic RNAase digestion products (see fig. 2b). The exact chain length of each fragment was then determined from the relative molar yield of its T_1 oligonucleotides. These results imply that the fragments were intact and absence of binding could not have been due to their degradation to shorter pieces.

Fig. 3 gives the sequence of 97 nucleotides which was determined by Weber [7] for the region preceding the Qβ coat cistron, and shows the location and sequence of each fragment tested. It is evident that a sequence of between 50–100 nucleotides preceding the initiator AUG is required for the formation of a 70 S complex. Fragments with less than 50 nucleotides (except F5), though protected and readily recovered from a preformed 70 S complex, do not rebind to ribosomes.

4. Discussion

Recently a nucleotide sequence has been determined for a ribosome binding site on φX174 DNA [13] and on the 0.3 gene transcription product of T7 DNA [14]. In each case the region just left of the initiation codon shows striking similarities to the R17 and Qβ RNA ribosome binding sites [15] which may in the future be explained in terms of ribosome and/or initiation factor specificity. For example, in φX174 DNA a sequence of eight nucleotides ending with the ATG initiator differs from the corresponding Qβ coat

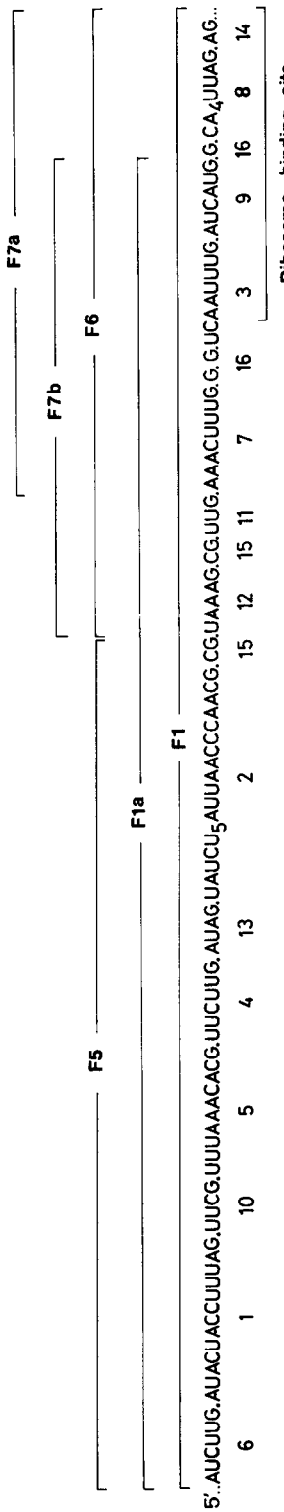

Fig. 3. Location of each fragment (F) at the Qβ coat cistron ribosome binding site. It is clear from the relative molar yield of T_1 oligonucleotides (not given) that gel band 7 contained a mixture of two fragments, 7a and 7b. The numbers beneath the sequence refer to the T_1 spots in fig. 2.

cistron ribosome binding site sequence by a single purine substitution. The lack of binding of fragments 6 and 7, both of which contain this sequence, therefore suggests that the sequences adjacent to initiation codons could only be part of a code for the initiation of proteins. Comparison of longer sequences preceding the cistrons of Qβ [3, 7] and R17 (or MS2) [4, 5, 17] does not, however, reveal any further sequence homology.

Absence of binding could be due to the isolated segment assuming a different conformation from that of the same region in the full-length messenger. Alternatively, a structural feature not present in the shorter segments may be required for binding. Information on this point could emerge from the binding of either formaldehyde treated fragments or chemically synthesized RNA segments of defined sequence.

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