ON THE POSSIBLE MODULATING ROLE OF THE ISOLEUCINE AUA-CODON IN BACTERIOPHAGE MS2 RNA

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SUMMARY

A set of MS2 mutants were shown to have an additional silent mutation met → ile at position 108 of the coat protein (13, 14). As transitions are more frequent than transversions one would have expected an AUA codon in this position in the mutant RNAs. As the AUA codon is one of the best candidates for a modulation role in the control of translation in E.coli, the presence of this AUA in the gene for the protein made in major amounts upon viral infection would impose serious doubt on the theory of modulation. We have directly proven by minifingerprinting of mutant RNA and further analysis of the relevant spots that, in fact, the isoleucine residue at position 108 of the coat protein gene is specified by the non-rate-limiting AUU codon, in agreement with a modulation type of control of protein synthesis.

According to the modulation theory (1,2) translation of an RNA message can be controlled by the proper choice of the code words which it contains. The presence of certain critical triplets would slow down the speed of translation and thus limit the amount of protein made in agreement with the needs of the cell. The direct dependence of rate of protein synthesis on the availability of tRNA has been shown in an Escherichia coli cell-free system with synthetic polynucleotides as messengers (3). It is also known that the level of different isoaccepting tRNAs varies widely in E.coli. Probably most relevant in this respect is the fact that the specific tRNAs corresponding to the codons AUA (4,5) and AGA/AGG (4) for ileucine and arginine respectively

are present in very low amounts. Furthermore, specialized cells synthesizing large amounts of only one or a limited number of proteins such as the posterior silk gland of Bombyx mori L. have been shown to adapt their tRNA population according to the major amino acids present in the proteins (6) which they synthesize. The rabbit reticulocyte glutamine isoaccepting tRNA species content is directly correlated with the presumed exclusive presence of CAG glutamine codons in the rabbit haemoglobin mRNA (7, 8).

The genome of group I RNA phages such as R17, f2 and MS2 specifies three proteins, namely the coat, the A-protein and the viral-coded replicase subunit. The mature virion contains 180 copies of coat protein and a single copy of A-protein, while the replicase has a catalytic function and is not incorporated in the virus particle. So it is clear that the three different gene products are required and in fact are made in very different amounts (9). Control mechanisms at the level of initiation of protein synthesis and of translational repression are known to act in this system (9) but this does not rule out a possible role of modulation in the achievement of the actual levels of proteins needed. Indeed, the unequal frequency of used code words in the three different genes, assigned from the known nucleotide sequence of the complete genome (10-12) can partly be interpreted in terms of a modulation-type control of translation, although other factors may also be important (12). Among the twelve codons absent in the coat protein gene (11) but used to specify the structure of the two other proteins, the best candidates as modulating codons are probably the isoleucine codon AUA and the arginine codons AGA/AGG (vide supra).

A set of MS2 mutants have been shown to contain an additional neutral amino acid substitution methionine → isoleucine at position 108 of the coat protein (13,14). Using our mini-fingerprinting procedure (15) we have now tried to answer the question : has the AUG codon been changed to an AUU, AUC or AUA codon ? A transition G → A would be most likely according to the variability observed among different RNA phages where transitions are highly predominant (16). However, the presence of an AUA codon in the coat protein gene would be incompatible with its possible role as a modulating codon and would impose serious doubt on the theory of modulation at least for procaryotic organisms. According to our analysis, however, the isoleucine residue at position 108 of the coat is coded for by the non-rate-limiting AUU codon, in agreement with a modulatory type of control of protein synthesis. The work presented here also demonstrates the usefulness of the mini-fingerprinting system for the comparative study between mutant and wild type form of even complex RNA molecules and between related RNAs in general.

MATERIALS AND METHODS

Conditions of mutagenesis, isolation and characterization of the MS2 mutants are beyond the scope of this article (13), and only a short description will be given here. The mutants were all derived from mutant am601 (17) which has an amber mutation at position 70 of the coat protein gene (Table 1). The mutant ts601 was isolated by selecting for ts revertants of the amber character. It has the wild-type glutamine residue at position 70 but contains a methionine → isoleucine change at position 108. Mutant ts1004am was obtained after nitrous acid mutagenesis of ts601 phage, while 1004am was isolated as a revertant of the ts character of ts1004am. Both mutants have maintained the isoleucine residue at position 108, showing that this substitution is almost certainly not responsible for the ts character of this set of mutants.

Both wild-type and the three mutant phages ts601, ts1004am and 1004am were grown in the presence of 5mC ^{32}P-phosphate. ^{32}P-labeled RNA was prepared from the purified virus by phenol extraction and had a specific radioactivity of 1-2 x 10^6 cpm/μg.

Table 1 : MS2 mutants used in this study

	mutation		
	ts	amber	(silent coat position 108)
MS2 wt	-	-	Met
↓ NH_2OH			
am 601	-	coat pos. 70	Met
↓ sp^x			
ts 601	+	-	Ile
↓ HNO_2			
ts 1004am	+	A-protein	Ile
↓ sp^x			
1004am	-	A-protein	Ile

x
sp means spontaneous

Approximately 30 µg of RNA was digested with ribonuclease T_1 and subjected to our mini-fingerprinting procedure (15). Regions of interest were characterized further by digestion with pancreatic ribonuclease and analysis on small PEI-thin layer plates (18).

RESULTS AND DISCUSSION

Upon visual inspection, the ribonuclease T_1 maps from the wild-type and the three mutant RNAs were indistinguishable (Fig. 1). In the wild-type RNA, the region surrounding the mutation site gives rise to the T_1 oligonucleotides CAAUG and CAAG (Table 2) both occurring several times in the MS2 RNA molecule (10-12). Mutation

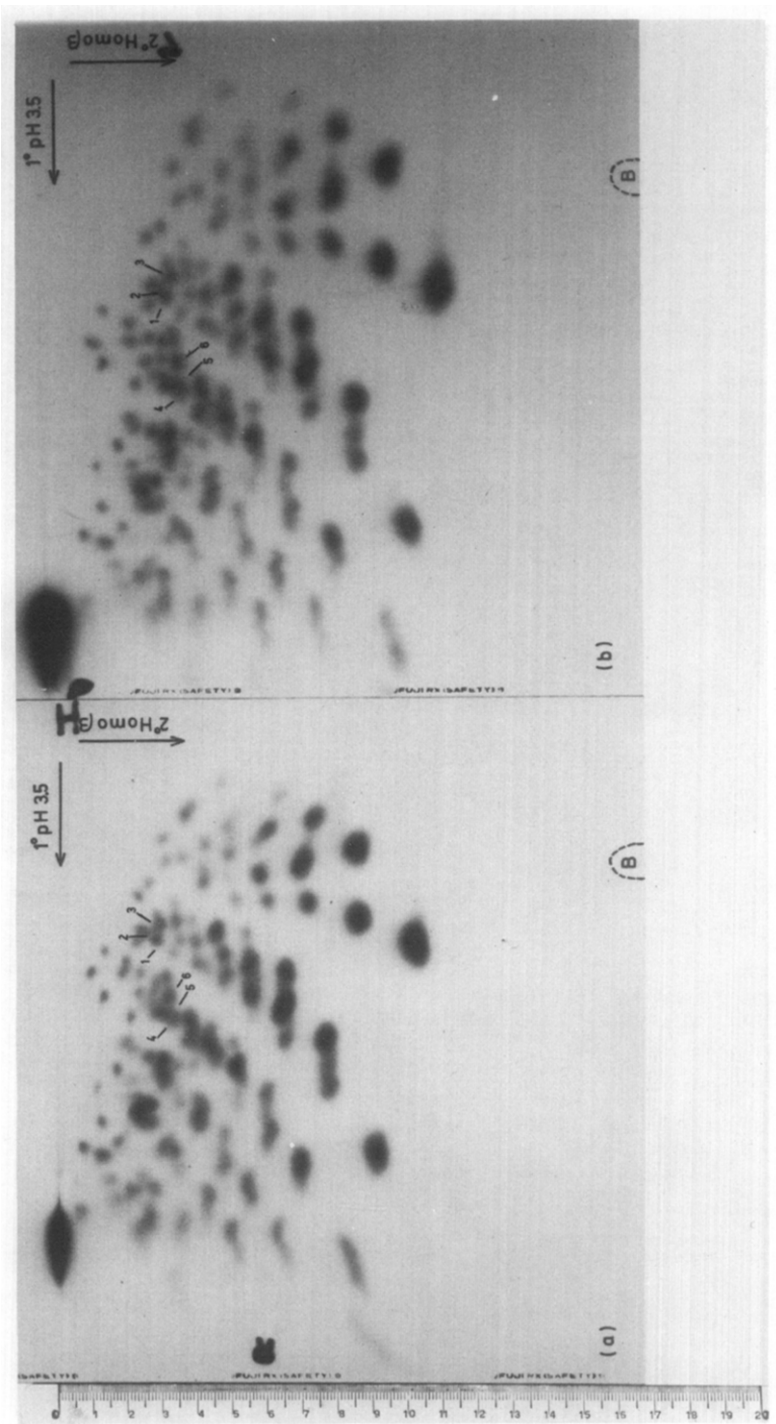

Figure 1 : Ribonuclease T_1 maps of wild type (a) and mutant ts 1004am RNA (b). Electrophoresis in the first dimension is on cellulose acetate at pH 3.5, development of the PEI-TL plate in the second direction is with RNA homomixture . B is the position of the xylene cyanol FF marker. The patterns from ts601 and 1004am (not shown) were also indistinguishable. Numbered spots have been analyzed further (Fig. 2 and Table 3). These preparations contained some contaminating DNA (dark spot in the upper left corner).

Table 2 : Ribonuclease T_1 oligonucleotides in the region of amino acids 107 - 110 of the coat protein

Source of RNA	Nucleotide sequence showing RNase T_1 cleavage points	Panc. composition of ribonuclease T_1 oligonucleotides involved	Panc. composition of sequence isomers present in the MS2 RNA molecule (a)	Nucleotide composition
Wild-type phage	.GCA.AUG.CAA.GGU.	-	-	-
Possibilities in mutant phages				
- transition G → A	.GCA.AUA.CAA.GGU.	$(C,AC,A_2U)A_2G$	-	125
- transversion G → C	.GCA.AUC.CAA.GGU.	$(C_3,A_2U)A_2G$	$(C_2,AU,A_2C)AG$ $(C_2,AC,A_3U)G$ $(U,C_2,AC)A_3G$	134
G → U	.GCA.AUU.CAA.GGU.	$(U,C_2,A_2U)A_2G$	$(U_2,C,AC)A_3G$	224

(a) Taken from a complete T_1 catalogue of MS2 RNA.

of the third letter of the methionine AUG codon leads to a nonanucleotide ribonuclease T_1 product. In the case of a G → A transition the product would have shown up as a new spot with composition 125 (15, footnote 1) if a C were present a component 134 would be formed, while a transversion G → U would result in a sequence 224. In the latter two instances (three, respectively one) sequence isomers are present in the MS2 RNA molecule (Table 2). However, the putative new oligonucleotide can be identified among the wild-type isomers on the basis of the products formed after pancreatic ribonuclease hydrolysis.

The regions on the fingerprint with presumed composition 125 - 134 - 143 and 224 - 233 were eluted, digested with pancreatic ribonuclease and analyzed on small PEI-plates (18), which allows easy identification of all possible double-digestion products. The presence of both A_2U and A_2G irrespective of the substitution which has occurred, should enable us to detect the putative new product even in a mixture of isomers derived from the rest of the molecule (Table 2).

An example of such an analysis (region 224 - 233 from mutant ts1004am) is shown in Fig. 2 and the results are summarized in Table 3. In the region 125 - 134 - 143 (spots 1-3) no qualitative differences were found between the four RNAs, thus excluding the presence of a mutant 125 or 134 product. It should be noted that one of the 134 isomers moves slightly slower in the first dimension and thus contaminates the adjacent 143 spot (spots 2 and 3). Analysis of spots 4-6, corresponding to nucleotide composition 224 - 233, however, revealed the presence of a new oligonu-

Footnote 1 : The numbers of the oligonucleotides refer to the adenosine (last figure), cytidine (middle figure) and uridine (first figure) content (19). All the products are 3'-Gp terminal.

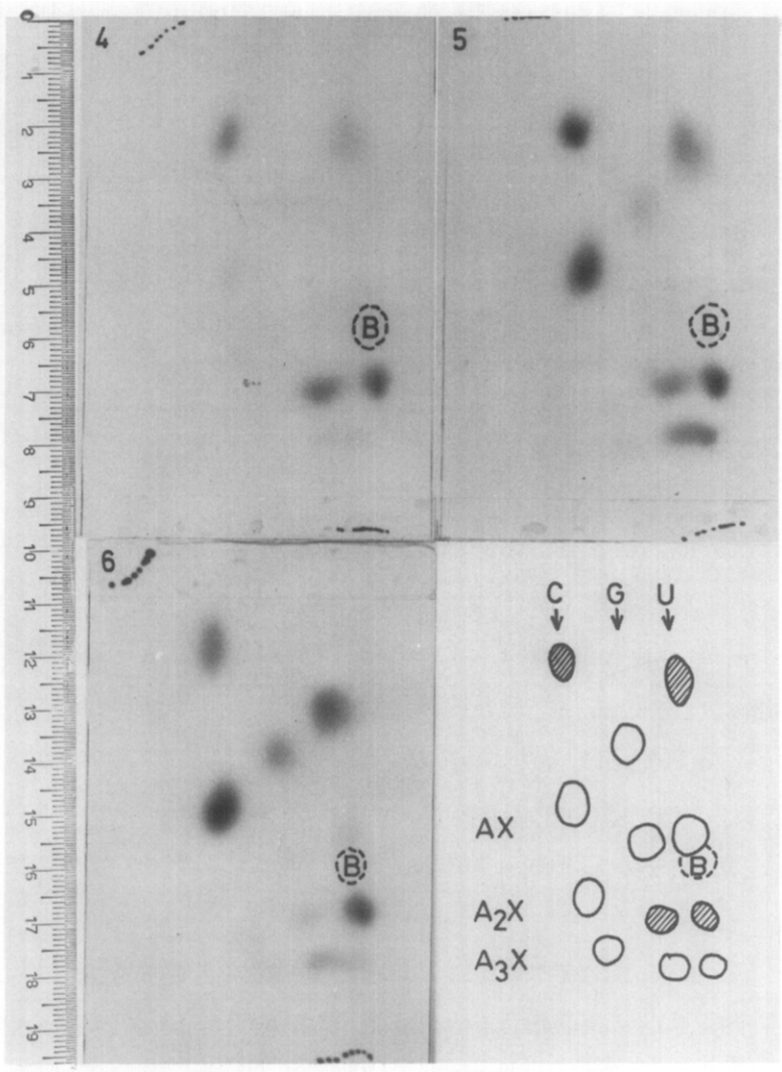

Figure 2 : Double digestion analysis of spots 4-6 corresponding to compositions no. 224 - 233 (Fig. 1). The material shown is derived from mutant ts1004am RNA. B is the position of the xylene cyanol FF marker. Spot no. 4 contains the sequence no. 224 $(U,C_2,A_2U)A_2G$ (contaminated with the wild-type isomeric sequence $(U_2,C,AC)A_3G$) in agreement with a G → U transversion in the mutants (Table 2), spot no. 5 contains a roughly equimolar mixture of both isomeric sequences, while spot no. 6 contains products corresponding to the MS2 sequences no. 233 $(U,C_2,AC,A_2U)G$ and $(U_2,(AC)_3)G$ (taken from a complete T_1 catalogue of MS2 RNA). In the wild-type MS2 RNA, the product $(U,C_2,A_2U)A_2G$ is absent. *Lower right-hand panel* illustrates the separation of the double digestion products : *according to C-, G- or U-content in the* first dimension and according to A-content in the second dimension. Shaded spots indicate products derived from the mutant oligonucleotide (C, U, A_2G and A_2U).

Table 3 : Pancreatic ribonuclease analysis of T_1 oligonucleotides

Spot no. (a)	Double digestion products present	Panc. composition of T_1 oligonucleotides deduced	Nucleotide composition
1. wild-type	C,AC,A_2C,G,AG,AU,A_3U	$(C_2,AU,A_2C)AG;(C_2,AC,A_3U)G$	134
mutants	idem	idem	idem
2. wild-type	C,AC,A_3C,G,A_3G,U	$(U,C_2,AC)A_3G;(U,C_3,A_3C)G$	134;143
mutants	idem	idem	idem
3. wild-type	C,A_3C,G,U	$(U,C_3,A_3C)G$	143
mutants	idem	idem	idem
4. wild-type	C,AC,A_3G,U	$(U_2,C,AC)A_3G$	224
mutants	C,AC,A_2G,A_3G,U,A_2U	$(U,C_2,A_2U)A_2G;(U_2,C,AC)A_3G$	224
5. wild-type	C,AC,A_3G,U	$(U_2,C,AC)A_3G;$	224
mutants	C,AC,A_2G,A_3G,U,A_2U	$(U,C_2,A_2U)A_2G;(U_2,C,AC)A_3G$	224
6. wild-type	C,AC,G,U,A_2U	$(U,C_2,AC,A_2U)G;\ U_2,(AC)_3\ G$	233
mutants	idem	idem	idem

(a) Spots are indicated in Fig. 1.

cleotide in the three mutant RNAs (Fig. 2). This means that a G → U transversion has occurred in the mutant (Tables 2 and 3). The mutant sequence 224 $(U,C_2,A_2U)A_2G$ moves slightly faster than the wild-type 224 isomer and in some experiments accounts for up to 80 % of the material in spot no.4.

In conclusion, we have shown that the neutral isoleucine amino acid change at position 108 of the coat protein is coded for by an AUU codon and not by the putative modulator codon AUA. Although a transition from AUG to AUA is a priori more likely to occur than a transversion to AUU, the presence of an AUA codon in the coat protein gene, however, would have been incompatible with its role as a modulating codon, and would have made the concept of modulation control of protein synthesis highly unlikely in the E.coli system. This study also illustrates the usefulness of the mini-fingerprinting system (14) for a variety of applications especially when rather complex RNA molecules have to be analyzed or compared.

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