INITIATION COMPLEX FORMATION WITH DOUBLE STRANDED RNA

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SUMMARY: Binding of formylmethionyltRNA and ribosomes to double stranded RNA has been obtained under conditions identical to those required for initiation complex formation with single stranded RNA. While natural double stranded RNAs from Penicillium chrysogenum virus and Penicillium stoloniferum virus were efficient in forming initiation complexes, the synthetic polynucleotide poly(I).poly(C) was inactive. This suggests that ribosomes can recognize initiation sequences even if these are present in base-paired form.

Initiation sites of protein synthesis have been characterized in a great number of viral RNAs (1-10) both as to their primary and secondary structure. The exact structural requirements for the recognition of these sites by ribosomes or by protein factors are, however, not yet known. Additional information needed to discriminate between the initiator codon and other AUG codons may be built into the primary or the secondary structure or into both. Early results on the hairpin loop structure of ribosome binding sites (1, 2, 8) suggested that a specific secondary structure may be required for recognition of the initiation site. In the light of recent observations it seems, however, that hairpin loops may not be needed for the formation of an initiation complex (11, 12, 13). There have been also earlier indications that a strong secondary structure may prevent rather than enhance initiation of protein synthesis (14, 15). The recently proposed mechanism of initiation which involves base pairing between a

pyrimidine-rich sequence near the 3'-end of 16 S ribosomal RNA and the purine-rich short sequences detected on the 5'-side of ribosome binding sites in most mRNAs (10, 12, 9) implies that strong secondary structure around the initiation site, far from being required for interaction with ribosomes, may rather interfere with it. In the case of 16 S RNA it has been suggested that ribosomal protein S1 may bring about the opening of a hair-pin loop with exposure of the ACCUCC sequence. (16). A similar conformational change has not been detected so far in mRNA, although recognition of the purine-rich sequence and annealing to 16 S RNA may require an open, single stranded RNA stretch. It is thus not clear at present in which way the secondary structure of mRNA participates in the initiation process: it may contribute to the formation of a recognition site or, on the contrary, may prevent attachment of ribosomes to mRNA. Abolition of a strong secondary structure might then become a prerequisite of initiation complex formation (11).

In order to study these possibilities we have chosen completely double stranded RNA as a model substance and assayed whether and under what conditions initiation complexes can be formed with such RNA molecules. RNAs from Penicillium chrysogenum and Penicillium stoloniferum viruses have been used. The former virus has been shown (17) to contain completely double stranded RNA, while P. stoloniferum virus contains both double stranded and single stranded RNA species (18, 19) and also single stranded stretches in the double stranded RNA molecules (20). All single stranded RNA components can be removed by RNAse T_1 treatment (21). The secondary structure of the double stranded RNAs is much stronger than that of the initiation sites of any mRNA and the double stranded structure is stable under the assay

conditions. As these double stranded RNAs represent the genetic material of two fungal viruses they can be expected to contain initiation sequences. The ability or disability of ribosomes to form initiation complexes with such RNA molecules should therefore shed light on the problem whether a base-paired structure prevents initiation of protein synthesis,

MATERIALS AND METHODS

MS2 RNA was a gift of Dr. B. Johnson. f2 RNA was isolated from f2 virus (Miles Laboratories). Double stranded RNA from P. chrysogenum virus was a gift of Dr. J. Edwardson. Double stranded RNA from P. stoloniferum virus was prepared as described earlier (20). Both double stranded RNA preparations were treated with RNAse T_1 to remove all single stranded components (21). Poly(I).poly(C) was a preparation of Miles Laboratories. Its melting profile showed a slight trailing indicating the presence of some single stranded material. The Tm was 66°C. After incubation with initiation factors, in the medium used for initiation assays, the trailing disappeared and the Tm did not change.

Crude initiation factor preparations, containing all three initiation factors, were obtained by ammonium sulphate fractionation of the S100 fraction of E. coli MRE 600 cells. Partial purification of the initiation factors was as follows: IF1, according to Lee-Huang and Ochoa (22) to Step 4; IF2 was either used as a crude ammonium sulphate fraction or purified by phosphocellulose chromatography according to Fakunding et al (23); IF3, according to Lee-Huang and Ochoa (24) to Step 4.

Initiation complex formation was assayed according to Meier et al. (25) with minor modifications.

RESULTS AND DISCUSSION

In a purified E. coli system initation complex formation was followed by measuring the initiation factor dependent binding of fmet-$tRNA_F^{met}$ in the presence of 70 S ribosomes and either double stranded (ds) or single stranded (ss) RNA. It was found that completely ds RNA from Penicillium chrysogenum virus or Penicillium stolonifereum virus can also form initiation complexes, although with a lower efficiency than the ss viral RNAs. As can

TABLE 1

Initiation complex formation with single stranded and double stranded RNA.

Exp.	RNA	Assay conditions	c.p.m	fmet-tRNA$_F^{met}$ binding due to added RNA c.p.m.
1.	None	Complete system	3523	
		no initiation factors	669	
	f2 (ss)	Complete system	12323	8800
		no initiation factors	806	137
	PcV (ds)	Complete system	5937	3414
		no initiation factors	646	0
2.	None	Complete system	3502	
		no IF3	1806	
	MS2 (ss)	Complete system	17330	13828
		no IF3	7957	6151
	PcV (ds)	Complete system	8956	5450
		no IF3	3057	1251
	poly (I).poly (C)	Complete system	4146	640
		no IF3	1516	0

Assay system according to Meier et al (25). RNA: 25 ug; initiation factors: in exp. 1, crude preparation containing all three factors, in exp. 2, IF1, IF2 and IF3 preparations purified partially as described in Methods. Total volume: 25 ul. Incubation: 20 min. at 24°C.
ss: single stranded RNA, ds; double stranded RNA, PcV: P. chrysogenum virus.

be seen in Table 1, fmet-tRNA$_F^{met}$ binding in the presence of ds RNA from P. chrysogenum virus was 28 - 39% of that obtained with f2 RNA or MS2 RNA. The lower efficiency may be due to the different secondary structure or may simply reflect a poorer interaction of E. coli ribosomes with heterologous RNA. With

ds RNA from P. stoloniferum virus the results were similar, although the binding was usually somewhat lower.

Initiation complex formation with ds RNA was absolutely dependent on initiation factors (exp. 1.). If partially purified preparations of initiation factors were used, some complex formation occurred in the presence of IF1 and IF2 only (probably because of contamination of one of these preparations with IF3) but a strong increase ensued upon addition of IF3 (exp. 2.).

If natural ds RNA was replaced by the synthetic polynucleotide poly (I). poly (C), no binding of fmet-$tRNA_F^{met}$ occurred. (exp. 2.). This suggests that the attachment of ribosomes and initiator tRNA to ds RNA is not an unspecific process but a specific binding which requires a suitable primary structure. It also implies that the primary structure is recognized even though the nucleotides are in base-paired form.

RNA preparations both from P. chrysogenum and P. stoloniferum virus have been pretreated with RNAse T_1 to remove ss RNA molecules (21) or ss RNA stretches (20). The effect observed is thus due to the ability of completely double stranded RNA to form initiation complexes. The requirements for complex formation are summarized in Table 2, they seem similar to the conditions of initiation with ss RNA. Further steps of translation do not take place if ds RNA is used as messenger. In a purified protein synthesizing system of E. coli (consisting of ammonium chloride-washed ribosomes, partially purified initiation factors, 250.000 g supernatant, supplemented with all the usual components required for translation) no amino acid incorporation was observed in the presence of P. chrysogenum viral RNA under conditions when f2 and MS2 RNA were actively translated.

TABLE 2

Requirements for initiation complex formation with double stranded RNA from P. chrysogenum virus.

Assay Conditions	c.p.m.
Complete system	4219
no IF3	2645
no ds RNA	2195
no ribosomes	748

Assay system as in Table 1.

It can be concluded that a strong secondary structure does not prevent the formation of initiation complexes, although it does prevent further steps in translation. Ribosomes can bind to double stranded structures and some characteristics of the primary structure can also be recognized while the nucleotides are in base-paired form. Whether any interaction occurs with the 3'-terminal part of 16 S RNA under these conditions, cannot be decided at present. The possibility may also be considered that one of the protein factors participating in the initiation process may specifically induce local unwinding of the double stranded structure at the site of interaction with ribosomes. Further work is in progress to study the possibility of such a mechanism.

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REFERENCES

1. Steitz, J.A. (1969). Nature 224:957-964.
2. Hindley, J. and Staples, D.H. (1969). Nature 224:964-967
3. Gupta, S.L., Chen, J., Schaefer, P., Lengyel, P. and Weissmann, S.M. (1970). Biochem. Biophys. Res. Comm. 39:883-888.
4. Staples, D.H. and Hindley, J. (1971). Nature New Biol. 234:211-212.
5. Staples, D.H., Hindley, J., Billeter, M.A and Weismann,C. (1971). Nature New Biol. 234:202-204.
6. Steitz, J.A. (1972). Nature New Biol. 236:71-75.
7. Volckaert, G. and Fiers, W. (1973). FEBS Letters 35:91-96.
8. Contreras, R., Ysebaert, M., Min Jou, W. and Fiers, W. (1973). Nature New Biol. 241:99-101.
9. Steitz, J.A. and Jakes, K. (1975). Proc. Natl. Acad. Sci. USA 72:4734-4738.
10. Shine, J. and Dalgarno, L. (1974). Proc. Natl. Acad. Sci. USA 71:1342-1346.
11. Steitz, A.J. (1973). Proc. Natl. Acad. Sci. USA 70:2605-2609
12. Shine, J. and Dalgarno, L. (1975). Nature 254:34-38.
13. Fiers, W., Contreras, R., Duerinck, F., Haegmean, G., Merregaert, J., Min Jou, W., Raeymakers, A. Volckaert, G., Ysebaert, M., Van de Kerckhove, J., Nolf, F. and Van Montagu, M. (1975). Nature 256: 273-278.
14. Lodish, H.F. (1970). J. Mol. Biol. 50:689-702.
15. Lodish, H.F. (1971). J. Mol. Biol. 56:627-632.
16. Dahlberg, A.E. and Dahlberg, J.E. (1975). Proc. Natl. Acad. Sci. USA 72:2940-2944.
17. Wood, H.A. (1973) J. Gen. Virol. 20:61-85.
18. Bozarth, R.F., Wood, H.A. and Mandelbrot, A. (1971). Virology 45:516-523.
19. Buck, K.W. and Kempson-Jones, G.F.(1973). J. Gen. Virol. 18:223-235.
20. Szekely, M. and Loviny, T. (1975). J. Mol. Biol. 93:79-87.
21. Loviny, T. and Szekely, M. (1973). Eur. J. Biochem. 35:87-94.
22. Lee-Huang, S. and Ochoa, S. (1974). "Methods in Enzymology" Moldave, K. and Grossman, L. eds., New York, N.Y., Academic Press, Vol. XXX., part F, pp. 31-39.
23. Fakunding, J.L., Traugh, J.A., Traut, R.R. and Hershey,J.W.B. (1974). "Methods in Enzymology", Moldave, K. and Grossman, L. eds., New York, N.Y., Academic Press, Vol. XXX., part F, pp. 24-31.
24. Lee-Huang, S. and Ochoa, S. (1974)."Methods in Enzymology", Moldave, K. and Grossman, L. eds., New York, N.Y., Academic Press, Vol. XXX., part F, pp. 45-53.
25. Meier, D., Lee-Huang, S. and Ochoa, S. (1973). J. Biol.Chem. 248:8613-8615.