Vol. 94, No. 1, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 14, 1980 Pages 218-225

STIMULATION OF AN ARTEMIA SALINA ATPase ACTIVITY BY EUKARYOTIC MESSENGER RNAs

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Received March 20,1980

SUMMARY: Several 'capped' mRNAs (e.g., 9S globin mRNA) as well as 'non-capped' mengo viral mRNA greatly stimulate the activity of a novel ATPase isolated from Artemia salina embryos. Other RNAs tested [tRNAs, initiator Met-tRNA, poly r(U), poly r(A)] are much less stimulatory. The ATPase activity is also stimulated by mRNAs in the presence of 40S ribosomal subunits. However, the other RNAs listed above are inhibitory under the latter conditions. The ATPase activity is strongly stimulated by 40S ribosomal subunits in the absence of mRNAs. It is suggested that the ATPase, described here, might be involved in the binding of natural eukaryotic mRNAs to 40S peptide chain preinitiation complexes, a process known to depend on ATP hydrolysis.

INTRODUCTION

Previous studies from other laboratories have shown that ATP is required for the binding of natural eukaryotic mRNAs (globin mRNA, TMV* RNA) to 40S ribosomal subunits during assembly of polypeptide chain initiation complexes (1-4). Furthermore, since the non-hydrolyzable analog AMPPCP cannot replace ATP in the above step, it is generally inferred that hydrolysis of ATP to ADP and P_i is required for maximal binding of mRNA to 40S subunits (2-4). However, the exact function of ATP in this reaction is not clear and nothing is known about the putative factor which plays a direct role in ATP hydrolysis during mRNA binding to 40S subunits. In this paper, we demonstrate that developing A. salina

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*Abbreviations: TMV, tobacco mosaic virus; AMV, avian myeloblastosis virus; AMPPCP, adenosine 5'-(β,γ-methylene)triphosphate; P_i, inorganic phosphate; MSF, messenger RNA-stimulated factor; eIF-2, eukaryotic initiation factor 2; $Met\text{-}tRNA_i$, initiator Met-tRNA.

0006-291X/80/090218-08$01.00/0

embryos contain a novel factor (MSF) whose ATPase activity is stimulated by natural eukaryotic mRNAs both in the absence and presence of 40S ribosomal subunits. To our knowledge, this is the first example of an enzymatic activity which is modulated by mRNAs. The possibility is discussed that MSF might be the putative factor which catalyzes ATP hydrolysis during the binding of natural mRNAs to 40S peptide chain preinitiation complexes.

MATERIALS AND METHODS

MSF was purified as follows. Development of A. salina embryos and preparation of 35-80% $(NH_4)_2SO_4$ fraction from high-salt ribosomal washes of developing embryos were carried out essentially as in (5). The $(NH_4)_2SO_4$ fraction was dialyzed and chromatographed successively on CM-Sephadex and DEAE-cellulose columns as described previously for eIF-2 purification (6). During purification, MSF activity was monitored by measuring the 40S ribosomal subunit-dependent ATPase activity (see Table 1). MSF of highest specific activity was eluted from DEAE-cellulose columns with buffer B (6) containing 70 mM KCl. This material was stored in small aliquots at -70° and used in this study. The following points should be noted: 1) MSF purified by the above procedure contains very little eIF-2; the latter factor is eluted from DEAE-cellulose columns with buffers of higher ionic strength (6). 2) MSF is purified ~10-fold by chromatography of the CM-Sephadex material on DEAE-cellulose; however, the overall purification (starting from the dialyzed $(NH_4)_2SO_4$ fraction) is likely to be considerably higher since most (~80%) of the protein present in the $(NH_4)_2SO_4$ fraction is not retained on CM-Sephadex columns while MSF is retained and is subsequently eluted along with only ~14% of the applied protein; the 40S ribosomal subunit-dependent ATPase activity is not detectable in the dialyzed $(NH_4)_2SO_4$ fraction presumably because of the presence of a large amount of non-specific ATPase and/or inhibitors in this fraction.

Ribosomal subunits were prepared from undeveloped A. salina embryos essentially as described previously (7) and were stored at -20° in a buffer containing 50% glycerol. As assayed by poly (U) translation, 40S subunits were essentially free of 60S subunits. However, 60S subunits were contaminated ~20% with the other species. Poly (A)-containing mRNA was isolated from developing A. salina embryos essentially as described by Grosfeld and Littauer (8). Total poly (A)-containing mRNA from rabbit reticulocytes and rat liver were kindly provided by Dr. M. Adesnik, New York University Medical Center. Pure 9S globin mRNA was a generous gift of Dr. M.J. Modak, Sloan Kettering Institute, New York; TMV, AMV and mengo virion RNAs were kindly provided by Dr. G.C. Sen, Sloan Kettering Institute, New York. As expected, there was no detectable ATPase activity in the mRNA preparations. Unlabeled Met-$tRNA_i$ was prepared from unfractionated calf liver tRNA essentially as described in (5). Other materials were obtained from the following companies: A. salina embryos (San Francisco Bay brine shrimp eggs, Metaframe) from Aquarium Stock Company, New York City, poly r(U), poly r(A) and AUG from Miles, unfractionated calf liver and yeast tRNAs from Boehringer Mannheim, bovine serum albumin from Sigma and [γ-^{32}P] ATP from ICN.

Unless mentioned otherwise, reaction mixtures (0.1 ml) for ATPase assay contained: Tris-HCl buffer, pH 7.4, 20 mM; Dithiothreitol, 1 mM; glycerol, 2% (v/v); KCl, 26 mM (Table 1) or 75 mM (Tables 2-4); Magnesium acetate, 5.5 mM (Table 1) or 2 mM (Tables 2-4); EDTA, 5 μM; albumin, 23 μg; ATP (labeled with ^{32}P in the gamma position), 3.0 μM. Samples, containing the above components only, were routinely used as blanks (the blank values were essentially the same in the presence or absence of albumin). Other additions are indicated in the legends to tables. Labeled ATP was added last and reaction mixtures were incubated for 15 min at 37°. The release of $^{32}P_i$ was measured essentially as described previously (9). Protein concentrations of MSF preparations were measured by ultraviolet absorption (10). RNA was estimated by using the relationship: 20 A_{260} units/mg of RNA/ml (11).

RESULTS

Purified MSF catalyzes only a low level of ATP hydrolysis in the absence of ribosomal subunits and mRNA (Tables 1-4). This ATPase activity is strongly stimulated by the addition of A. salina 40S ribosomal subunits in the absence of mRNA (Tables 1, 3, 4). ATP hydrolysis which requires the presence of both MSF and ribosomal subunits was calculated as follows: the sum of the activi-

Table 1

Effect of ribosomal subunits on ATPase activity of MSF

Additions	Net release of $^{32}P_i$ (pmol)	MSF- and ribosome-dependent ATPase* (pmol)	$\frac{40S}{40S + 60S}$
40S	4.43	-	
60S	1.00	-	
40S + 60S	5.69	-	
MSF	2.76	-	
" + 40S	36.30	29.11	
" + 60S	23.95	20.19	
" + 40S + 60S	45.20	36.75	0.79

* See Results for a description of this calculation.

Conditions of ATPase assay (see Methods). Other additions (in the order listed) were: 40S subunit (when present), 0.1 A_{260} unit; 60S subunit (when present), 0.23 A_{260} unit and MSF, 1.2 μg. A blank value of 7.29 pmol has been subtracted. The specific radioactivity of [γ-^{32}P] ATP was 2569 cpm/pmol. The values given are average of duplicate samples.

ties observed with subunits alone and MSF alone was subtracted from the activity observed upon combination of the above components (Table 1). The results indicate that MSF- and ribosome-dependent ATP hydrolysis (Table 1) occurs predominantly with 40S subunits. The stimulatory effect of 60S subunits is less than that of 40S subunits and is probably due to substantial contamination of the 60S subunit preparation with 40S subunits (see Methods). The stimulation of ATPase activity resulting from the addition of both 40S and 60S subunits to MSF is less than their calculated additive effect. These results, along with the fact that the 40S subunits are essentially free of 60S subunits as judged by poly (U) translation, indicate that the ability of isolated 40S subunits to stimulate the ATPase activity of MSF is not due to contamination with 60S subunits.

The ATPase activity of MSF is greatly stimulated (13-30 fold) by low concentrations of total poly (A)-containing mRNAs from A. salina, reticulocyte and rat liver (Tables 2-4). This stimulatory effect is dependent on the concentration of A. salina mRNA (Table 2). Similar results were obtained using varying

Table 2

Effect of different concentrations of A. salina mRNA on ATPase activity of MSF

Additions	Net release of $^{32}P_i$ (pmol)	Stimulation by mRNA (pmol)	(fold)
MSF	2.34	-	-
" + mRNA (0.31 μg)	10.37	8.03	4.4
" + mRNA (0.62 ")	37.95	35.61	16.2
" + mRNA (1.25 ")	43.42	41.08	18.6

Conditions of ATPase assay (see Methods). Other additions (in the order listed) were: the indicated amounts of A. salina mRNA (when present) and MSF, 1.2 μg. A blank value of 5.89 pmol has been subtracted. The specific radioactivity of [γ-^{32}P] ATP was 2032 cpm/pmol.

Table 3

Effect of various mRNAs on ATPase activity of MSF in the absence and presence of 40S ribosomal subunits

Expt. No.	Additions		Net release of $^{32}P_i$ (pmol)	Stimulation by mRNA (pmol)	Stimulation by mRNA (fold)
1	MSF		1.37*	-	-
	" + A. salina mRNA	(0.62 μg)	26.84	25.47	19.6
	" + reticulocyte mRNA	(0.90 ")	17.46	16.09	12.7
	" + rat liver mRNA	(0.91 ")	19.74	18.37	14.4
2	MSF		1.15*	-	-
	" + A. salina mRNA	(0.62 ")	31.51	30.36	27.4
	" + globin mRNA	(0.50 ")	18.98	17.83	16.5
	" + " "	(1.00 ")	15.70	14.55	13.7
	" + mengo RNA	(2.00 ")	29.38	28.23	25.5
	" + " "	(5.00 ")	23.78	22.63	20.7
	" + AMV RNA	(2.00 ")	24.59	23.44	21.4
	" + " "	(5.00 ")	22.46	21.31	19.5
	" + TMV RNA	(1.50 ")	20.58	19.43	17.9
	" + " "	(3.50 ")	32.68	31.53	28.4
3	MSF + 40S		18.82*	-	-
	" + " + A. salina mRNA	(1.25 ")	43.04	24.22	2.3
	" + " + rat liver mRNA	(1.25 ")	32.96	14.14	1.8
	" + " + reticulocyte mRNA	(1.25 ")	27.93	9.11	1.5
	" + " + globin mRNA	(0.62 ")	27.90	9.08	1.5
	" + " + mengo RNA	(3.00 ")	32.47	13.65	1.7
	" + " + AMV RNA	(3.00 ")	44.73	25.91	2.4
	" + " + TMV RNA	(2.00 ")	38.71	19.89	2.1

* Average of duplicate samples.

Conditions similar to those of Table 2. Other additions (in the order listed) were: the indicated amounts of various mRNAs (when present), 0.1 A_{260} units of 40S ribosomal subunits (when present) and MSF, 1.2 μg. Blank values (pmol), averaging 6.35 (expt. 1), 5.91 (expt. 2) and 5.58 (expt. 3), have been subtracted. The specific radioactivity of [γ-^{32}P] ATP (cpm/pmol) was 1929, 1821 and 1750 in expts. 1, 2 and 3, respectively.

concentrations of reticulocyte and rat liver mRNA (data not shown). Pure 9S globin mRNA and three viral mRNAs were also assayed at two levels for their ability to stimulate the ATPase activity of MSF. The results are given in Table 3 (expt. 2). All of these mRNAs are highly (~17-28 fold) stimulatory. Except for TMV RNA, the lower concentration of each of the other three RNAs seems to give maximal stimulation. Since mengo RNA lacks a 'cap' structure

at the 5'-end (12), it would appear that both 'capped' and 'noncapped' mRNAs stimulate the ATPase activity.

The ATPase activity, observed in the presence of MSF plus 40S ribosomal subunits, is also stimulated by mRNAs (Table 3, expt. 3). Although the fold stimulation (~1.5-2.5 fold) is not as striking under these conditions, the addition of various mRNAs still causes a substantial increase (~9-26 pmol) in the MSF-dependent ATPase activity. mRNAs have little or no effect on the ATPase activity due to 40S subunits alone (data not shown).

A comparison of the effect of other RNAs with that of mRNA on the ATPase activity of MSF is shown in Table 4. In the absence of 40S subunits, all of the other RNAs tested stimulate the ATPase activity to a relatively small extent (~2.3-3.7 fold). However, the stimulation by A. salina mRNA under identical conditions is much higher (~26-34 fold). The addition of the initiator codon AUG causes little or no increase in the ATPase activity (Table 4, expt. 1). Similar results were obtained when AUG and the other RNAs were tested at lower concentrations (data not shown). A striking difference between mRNA and other RNAs is also apparent when their effects are tested on the ATPase activity observed in the presence of MSF plus 40S ribosomal subunits (Table 4, expts. 1, 3). While A. salina mRNA stimulates ~2-fold under these conditions, the addition of AUG and other RNAs results in a 30-66% inhibition. Although the ATPase activity due to MSF alone or MSF plus 40S subunits varies somewhat in different experiments, the pronounced stimulatory effect of A. salina mRNA is observed in each experiment.

DISCUSSION

The preceding results indicate that the ATPase activity of MSF is stimulated by both natural mRNAs and 40S ribosomal subunits. This, in turn, suggests that the putative factor, which catalyzes ATP hydrolysis during the bind-

Table 4

Effect of various RNAs and AUG on ATPase activity of MSF in the absence and presence of 40S ribosomal subunits

Expt. No.	Additions		Net release of $^{32}P_i$ (pmol)	Effect of RNA: Stimulation (fold)	Effect of RNA: Inhibition (%)
1	MSF		1.43*	-	-
	" + A. salina mRNA	(1.25 μg)	48.40	33.8	-
	" + poly r(U)	(9.60 ")	5.11	3.6	-
	" + poly r(A)	(9.60 ")	4.50	3.1	-
	" + Met-$tRNA_i$	(7.80 ")+	4.67	3.3	-
	" + AUG	(0.05 A_{260})	1.63	1.1	-
	MSF + 40S		25.52*	-	-
	" + " + A. salina mRNA	(1.25 μg)	51.21	2.0	-
	" + " + poly r(U)	(9.60 ")	10.81	-	58
	" + " + poly r(A)	(9.60 ")	14.44	-	43
	" + " + Met-$tRNA_i$	(7.80 ")+	9.66	-	62
	" + " + AUG	(0.05 A_{260})	17.70	-	31
2	MSF		0.77*	-	-
	" + A. salina mRNA	(0.75 μg)	19.74	25.6	-
	" + yeast tRNA	(2.00 ")	1.80	2.3	-
	" + yeast tRNA	(7.50 ")	2.86	3.7	-
3	MSF + 40S		13.57*	-	-
	" + " + A. salina mRNA	(1.25 ")	25.70	1.9	-
	" + " + calf liver tRNA	(7.80 ")	8.35	-	38
	" + " + yeast tRNA	(7.50 ")	4.58	-	66

*Average of duplicate samples.
+Contained ~2.5 pmol of the acylated species.

Conditions similar to those of Table 3. Other additions (in the order listed) were: the indicated amounts of the various RNAs or AUG (when present), 0.1 A_{260} units of 40S ribosomal subunits (when present) and MSF, 1.2 μg. Blank values (pmol) of 6.53 (expt. 1), 5.0 (expt. 2) and 5.10 (expt. 3) have been subtracted. The specific radioactivity of [γ-^{32}P] ATP (cpm/pmol) was 2120, 1575 and 1510 in expts. 1, 2 and 3, respectively.

ing of natural mRNAs to 40S ribosomal subunits (2-4), might be MSF. It should be pointed out that purified MSF preparations also contain a GTPase activity which is stimulated by 40S ribosomal subunits, by mRNA, and by mRNA in the presence of 40S subunits (data not shown). At subsaturating substrate concentrations, the 40S subunit- or mRNA-stimulated ATPase activity is ~1.5-2 fold greater than the corresponding GTPase activity (data not shown). However, it

is important to emphasize that the ATPase (and GTPase) activities observed under these conditions are uncoupled from the stable binding of natural mRNAs to 40S ribosomal subunits since the latter process is known to require several initiation factors and other components in addition to ATP (2-4). Thus, although GTP may replace ATP in the uncoupled reactions, complexes containing ATP (e.g., ATP·MSF complex) might be much more efficient than the corresponding GTP-containing complexes in promoting the stable binding of natural mRNAs to 40S ribosomal subunits during polypeptide chain initiation. The hydrolysis of ATP (originally present in the ATP·MSF complex, for example) may be coupled to this mRNA binding and may serve to release MSF from 40S initiation complexes

ACKNOWLEDGEMENTS: This work was aided by a grant from the National Science Foundation (PCM 77-25448). We are grateful to Drs. M. Adesnik, M.J. Modak, and G.C. Sen for their gifts of mRNAs. We thank Dr. W. Szer for the poly r(A) sample.

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