Biochimica et Biophysica Acta, 773 (1984) 308–316
Elsevier

BBA 72140

THE ROLE OF PROTEIN PHOSPHOKINASE AND PROTEIN PHOSPHATASE DURING THE NUCLEAR ENVELOPE NUCLEOSIDE TRIPHOSPHATASE REACTION

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(Received February 2nd, 1984)

Key words: Nuclear membrane; Nucleoside triphosphatase; Protein phosphokinase; Protein phosphatase; mRNA transport; (Quail oviduct; Quail liver)

The activities of nuclear envelope-associated protein phosphokinase and protein phosphatase were determined in nuclear ghosts from liver and oviduct of quails. The protein kinase was found to be inhibited by poly(A) by 75%. During the kinase reaction proteins with molecular weights of 106000 and 64000 were phosphorylated. The phosphoprotein phosphatase from liver was stimulated to 190% by poly(A), whereas only a slight enhancing effect by this polymer was determined with the oviduct enzyme (to 125%). Comparative determinations of the nuclear ghost-associated enzyme activities revealed the following values (in nmol P_i/min per 10^8 ghosts); oviduct: phosphokinase, 0.015; phosphatase, 0.004 and nucleoside triphosphatase, 39.4; and liver: phosphokinase, 0.044; phosphatase, 0.012 and nucleoside triphosphatase, 11.7. These data indicate that phosphorylation/dephosphorylation proceeds independently of the nucleoside triphosphatase cycle. This assumption is supported by analytical results revealing that no marked dephosphorylation occurs after poly(A) binding to the nuclear envelope. Moreover, stoichiometrical data showed a nearly 1:1 molar ratio between ATP-binding and phosphorylation of nuclear envelope protein. From these findings a new model for the nucleoside triphosphatase-mediated poly(A)(+)mRNA efflux from nuclei is deducted, proposing phosphokinase and phosphatase only to modulate the affinity of the 'carrier structure' for poly(A)(+)mRNA, but not to constitute the nucleoside triphosphatase.

Introduction

The following evidence strongly suggests that the nucleoside triphosphatase, which is associated with the nuclear envelope, plays a major role in the control of mRNA translocation through the nuclear pore complex: (a) In vitro, nucleocytoplasmic mRNA transport is an energy-requiring process [1], which is inhibited proportionally to the nucleoside triphosphatase activity by quercetin and [γ-thio]ATP [2]; (b) a correlation between alterations in mRNA transport and nuclear envelope nucleoside triphosphatase activity was demonstrated [3,4]; (c) antibodies directed against nuclear envelopes substantially reduced ATP-dependent release of RNA from rat liver nuclei [5]. The transported RNA must have an organized tertiary structure [6], in whose stabilization poly(A) plays a major role [7,8]; the unidirectional transport of mRNA is assumed to be controlled by poly(A)-associated protein(s) [8] and perhaps by microtubule protein [8,9]. Additionally to the nucleoside triphosphatase activity, the nuclear envelope is associated with both a protein kinase and a phos-

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Abbreviations: Polynucleotides and nucleotides are abbreviated according to the recommendations of the Commission on Biochemical Nomenclature; see (1970) Eur. J. Biochem. 15, 203–208. Enzymes: nucleoside triphosphatase (EC 3.6.1.15); protein kinase (EC 2.7.1.37); protein phosphatase (EC 3.1.3.?).

phoprotein phosphohydrolase activity [10], which were thought to constitute together the 'nucleoside triphosphatase' [2]. Following this model [1], phosphorylation and dephosphorylation of a 'carrier structure' are the essential steps in the nucleoside triphosphatase-mediated nucleocytoplasmic mRNA efflux from nuclei. However, in the course of our studies it became obvious that phosphorylation and dephosphorylation of the 'carrier structure' are processes which occur independently of the nucleoside triphosphatase reaction. Based on experimental results and on stoichiometrical calculations, a new model of mRNA efflux from nuclei is proposed. In this scheme, ATP binding to the phosphorylated protein induces a conformational change of the 'carrier structure', which facilitates the release of the bound mRNA. The phosphorylation/dephosphorylation cycle is thought to be only involved in the modulation of the affinity of this 'structure' to mRNA; no evidence exists that these processes are part of the mRNA translocation cycle itself.

Materials and Methods

Materials. [γ-^{32}P]ATP (spec. act. 3 Ci/mmol), [^{3}H]poly(A) (12 Ci/mmol of phosphate) (M_r 12500–45000) and [2,8-^{3}H]ATP (31 Ci/mmol) were obtained from the Radiochemical Centre (Amersham, England); *O*-phospho-DL-serine from Sigma (St. Louis, MO); poly(A) (single-stranded; with an average M_r of 248000 as determined by Studier [11]) and ATP (No. 127531) from Boehringer (Mannheim, F.R.G.); heparin (No. 24590) from Serva (Heidelberg, F.R.G.).

Animals. If not stated otherwise, livers or oviducts from immature female quails (*Coturnix coturnix coturnix*) have been used, which had been received 6 days diethylstilbestrol [12,13].

Nuclear ghosts and pore-laminae. Nuclear ghosts were prepared from oviduct and liver as described by Kaufmann et al. [14] and outlined earlier [7]. Nuclear ghosts were suspended at a concentration of $5 \cdot 10^8$/ml. Pore-laminae were prepared from nuclear ghosts as described by Dwyer and Blobel [15].

Protein phosphokinase assay. The kinase reaction was carried out according to McDonald and Agutter [10] with modifications. The standard assay mixture (final volume of 100 μl) contained 25 mM Tris-HCl buffer (pH 8.0), 1 mM 2-mercaptoethanol, 2.5 mM $MgCl_2$, 150 mM NaCl, 10 mM NaF, 10 mM *O*-phospho-DL-serine, 40 μM [γ-^{32}P]ATP (about 1500 dpm per pmol) and $5 \cdot 10^6$ nuclear ghosts. To some assays poly(A) was added; the exact concentrations are given in the experimental section. The incubation was performed routinely at 20 °C for 20 min. The reaction was terminated by precipitation with 1 ml of cold 5% trichloroacetic acid (containing 1.5% sodium pyrophosphate) followed by three washes with 5 ml of 5% trichloroacetic acid. The precipitated protein was counted in 10 ml of Aquasol (NEN).

Protein phosphatase assay. Nuclear ghosts were phosphorylated (20 °C; 60 min) in the protein phosphokinase reaction mixture (100 μl) in the presence of 40 μM [γ-^{32}P]ATP as described above; *O*-phospho-DL-serine and poly(A) were omitted from the reaction mixture. The reaction was terminated by addition of 900 μl of ice-cold 50 mM Tris-HCl buffer (pH 8.0), supplemented with 10 mM ATP, 1 mM $MgCl_2$ and 10 mM NaF. After centrifugation (12000 × *g*; 5 min; 2 °C), the sediment was suspended in 1 ml of the same Tris-HCl buffer, but without NaF, and centrifuged again. After an additional wash using 10 mM Tris-HCl (pH 8.0; 1 mM $MgCl_2$), the phosphorylated ghosts were suspended at a concentration of $2.5 \cdot 10^7$ ghosts/ml in a 50 mM histidine-imidazole buffer (pH 7.3; 1 mM $MgCl_2$) (10) and incubated routinely at 20 °C for 20 min. Where indicated, poly(A) was added to the reaction mixtures. Subsequently, 200 μl aliquots were withdrawn supplemented with 800 μl 50 mM Tris-HCl (pH 8.0), 1 mM $MgCl_2$, 10 mM NaF and 10 mM ATP, and chilled to 0 °C. Then, the suspension was centrifuged (12000 × *g*; 5 min; 2 °C), and the pellet was collected for determination of radioactivity.

Gel electrophoresis. Polyacrylamide gel electrophoresis was performed according to Weber and Osborn [16]. The protein kinase reaction was terminated by incubation at 95 °C for 5 min. Subsequently, sodium dodecyl sulfate (final concentration, 2%) and 2-mercaptoethanol (0.1%) were added. After heating for 2 min at 100 °C, samples of 200 μl were applied to 10% polyacrylamide gels, containing 0.1% sodium dodecyl sulfate. Electrophoresis was carried out in cylindrical gels [9]. The

gels were cut into 1-mm slices, and the radioactivity was determined as described [17]. The gels were calibrated by protein standards which were run in parallel [9].

Protein was determined by the method of Lowry et al. [18] using bovine serum albumin as standard. ATP, ADP and AMP were quantitatively analyzed by ion-exchange chromatography [19].

Results

Protein phosphokinase

The determinations were performed under conditions previously found to be optimal for this enzyme [10,20]. In contrast to the nucleoside triphosphatase [2], the activity of protein phosphokinase was stimulated in the presence of higher ionic strength; maximal activity was achieved at 150 mM NaCl (Fig. 1). Besides NaF, we included also *O*-phospho-DL-serine [21] into the assay mixture in order to suppress protein phosphatase activity (Fig. 2). Under these conditions, the kinase reaction was linear during the first 20 min of incubation (Fig. 2). Optimal incorporation occurred at 20 °C; the temperature coefficient Q_{10} 20 °C/10 °C was 4.6, and the one 20 °C/30 °C was 5.3.

Incubation of nuclear ghosts under these conditions resulted in phosphorylation of intrinsic membrane proteins as analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis. Polypeptides of M_r 64000 (P64) and M_r 106000 (P106) were phosphorylated during the reaction (Fig. 3). Poly(A) caused an inhibition of the kinase in all nuclear ghost samples tested (Table I). Dose-response experiments revealed a 50% inhibition in the presence of 20 μM poly(A) (with respect to phosphate content; data not shown). Selecting a poly(A) concentration of 90 μM, the inhibition of the oviduct enzyme was 71% and the reduction of the activity of the liver enzyme 73%. It could be demonstrated that phosphorylation of both P64 and P106 was differentially inhibited in the presence of poly(A) (Fig. 3 and Table II). Based on polyacrylamide gel electrophoresis, inhibition of phosphorylation of P64 to 50% was obtained at approx. 50 μM of poly(A), while a 50% reduction of P106 phosphorylation occurred already at about 3 μM. As shown in Fig. 1 poly(A) had no influence on the modulating effect of different NaCl concentrations on the enzyme activity. Furthermore, heparin, a known inhibitor of nucleoside

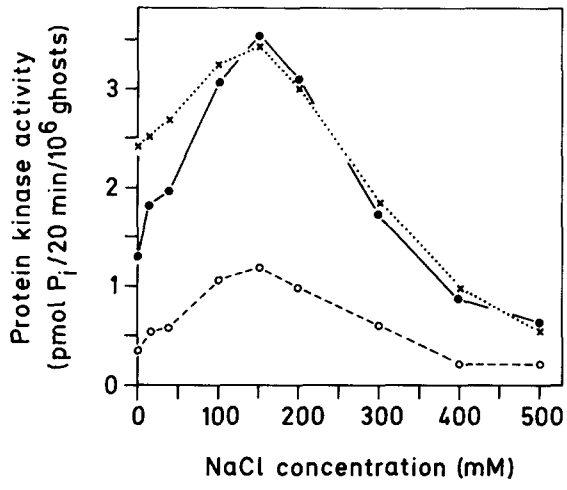

Fig. 1. Effects of different NaCl concentrations on protein kinase activity. Nuclear ghosts from oviducts of quails were used in the standard assay; incubation period, 20 min. Different NaCl concentrations in the absence of poly(A) (×·····×), or in the presence of poly(A) (90 μM P_i) (O------O) or of 0.1 μg/ml heparin (●———●).

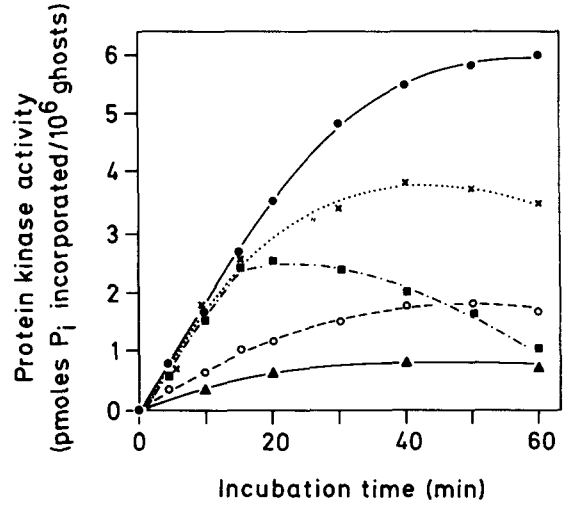

Fig. 2. Time-course of protein kinase reaction. Nuclear ghosts from quail oviduct were used in the standard protein phosphokinase assay. Standard assay conditions at 20 °C (with 10 mM phosphoserine) in the absence (●———●) or the presence of 90 μM poly(A) (O------O) . Reactions (without poly(A)) carried out in the presence of 0 (■·-·-·■) or 1 mM phosphoserine (×·····×). Reaction performed in the presence of 10 mM phosphoserine and at 30 °C (▲———▲).

TABLE I

LEVELS OF PROTEIN KINASE AND PROTEIN PHOSPHATASE ACTIVITY IN NUCLEAR GHOST PREPARATIONS FROM OVIDUCT AND LIVER

The experiments were performed under standard conditions (20 min incubation period) in the absence or presence of poly(A) (90 μM phosphate). The results of five experimental series are given (mean $\pm$ S.D.).

Tissue	Protein kinase activity (pmol P_i/20 min per 10^6 ghosts)		Protein phosphatase activity (pmol P_i/20 min per 10^6 ghosts)	
	minus poly(A)	plus poly(A)	minus poly(A)	plus poly(A)
Oviduct	3.53 $\pm$ 0.21	1.03 $\pm$ 0.09	2.09 $\pm$ 0.19	2.62 $\pm$ 0.22
Liver	8.98 $\pm$ 0.74	2.39 $\pm$ 0.20	2.22 $\pm$ 0.21	4.16 $\pm$ 0.33

triphosphatase activity in nuclei [22], showed no inhibitory influence on kinase activity (Fig. 1); at low salt concentrations this polyanion stimulated the phosphate incorporation by 85%.

Protein phosphatase

The kinetics of phosphorylation of nuclear envelope protein showed no lag phase and was linear under the assay conditions used during the first 20 min (Fig. 4). After that period of time, the curve levels off and reaches a plateau after 60 min with 3.8 pmol P_i released per 10^6 ghosts from oviducts. Using ghost preparations from the same tissue (Fig. 2), a maximal phosphorylation of 5.9 pmol P_i incorporation per 10^6 ghosts occurred during a 60-min incubation period. This means that in the standard assay only a partial dephosphorylation of the proteins took place. One factor which can contribute to a more complete dephosphorylation was found to be poly(G) [10]; this homoribopolymer increased the extent of dephosphorylation by 30%. In our studies, using poly(A), only a 7% stimulation of the phosphatase activity was obtained after 60 min (Fig. 4); however, at an incubation period of 20 min, poly(A) stimulated

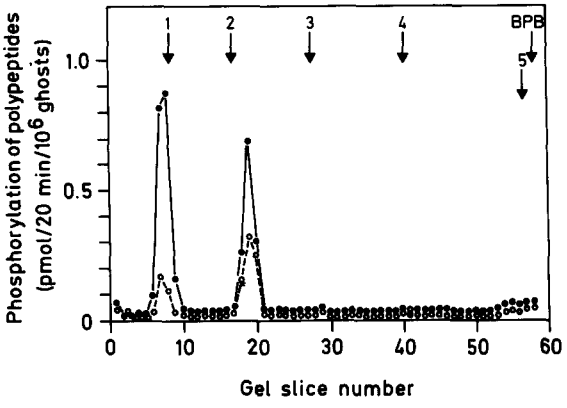

Fig. 3. Influence of poly(A) on phosphorylation of polypeptides of nuclear ghosts from quail oviduct. Phosphorylation of nuclear ghosts from oviducts of quails was performed in the standard protein kinase assay in the absence (●——●) or in the presence of 86 μM of poly(A) (○------○). Subsequently the samples were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis. The gels were sliced, and the radioactivity was determined as described under Materials and Methods. The arrows mark the positions of the following protein standards which were run in parallel gels: 1, phosphorylase *a* (M_r = 98000); 2, bovine serum albumin (M_r = 68000); 3, ovalbumin (M_r = 43000); 4, chymotrypsinogen *a* (M_r = 25000); 5, cytochrome *c* (M_r = 12000); BPB, Bromophenol blue.

TABLE II

INHIBITION OF POLYPEPTIDE-PHOSPHORYLATION BY POLY(A)

Nuclear ghosts from quail oviducts were phosphorylated in the standard protein kinase assay in the presence of increasing concentrations of poly(A). Nuclear envelope proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis. The extent of phosphorylation is given both in absolute and in percental values. Mean values of five experimental series are given; the S.D. varies between 6 and 10%.

Poly(A) concn. (μM phosphate)	Phosphorylation of polypeptides (pmol/20 min per 10^6 ghosts; or percent)	
	P106	P64
0	1.97 (61%)	1.26 (39%)
2.9	1.09 (47%)	1.22 (53%)
8.6	0.61 (34%)	1.17 (66%)
29	0.49 (40%)	0.74 (60%)
86	0.31 (38%)	0.51 (62%)
290	< 0.05	< 0.05

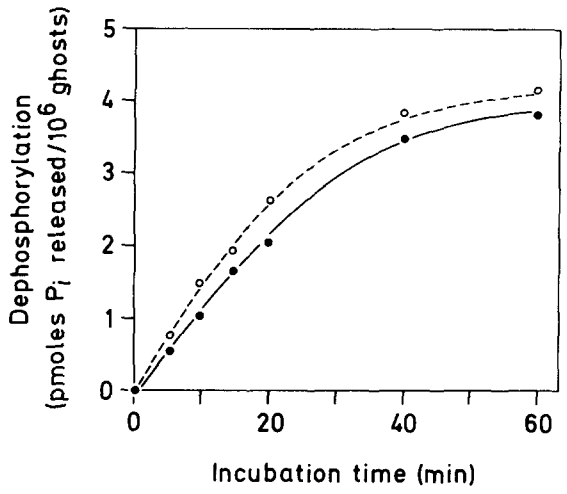

Fig. 4. Dephosphorylation of phosphorylated nuclear ghosts. Phosphorylation of nuclear ghosts from quail oviducts was performed in the standard protein phosphokinase assay. Dephosphorylation was carried out in the absence (●——●) or presence of 90 μM poly(A) (O------O) as described under Materials and Methods. The radioactivity released from the ^{32}P-labelled nuclear ghosts is indicated.

protein phosphatase by 25% (Fig. 4; Table I). The basal levels of enzyme dephosphorylation were determined to be higher in liver compared to oviduct (Table I). Poly(A) stimulated the protein phosphatase activity of liver to a higher extent (to 187%) compared to the stimulation of activity associated with oviduct ghosts (to 125%; Table I).

TABLE III

COMPARISON OF THE NUCLEAR ENVELOPE-ASSOCIATED PROTEIN PHOSPHOKINASE, PROTEIN PHOSPHATASE AND NUCLEOSIDE TRIPHOSPHATASE ACTIVITIES

Ghosts from mature quails were used for the experiments; the enzyme reactions were performed in the absence of poly(A). The nucleoside triphosphatase data are taken from Ref. 12.

	Activity (nmol P_i/min per 10^8 ghosts)		
	Protein phosphokinase	Protein phosphatase	Nucleoside triphosphatase
Oviduct	0.015	0.004	39.4
Liver	0.044	0.012	11.7

Nucleoside triphosphatase reaction

In a first attempt to clarify whether the nuclear envelope associated protein kinase and protein phosphatase are involved in the overall nucleoside triphosphatase reaction, a comparison of their activities was performed (Table III). Using oviducts and livers from quails, the activity of the protein

TABLE IV

INFLUENCE OF POLY(A) AND/OR ATP ON THE P_i RELEASE FROM NUCLEAR GHOSTS

$2.5 \cdot 10^7$ nuclear ghosts from liver of mature animals were incubated in the standard protein phosphokinase assay in the presence of [^{32}P]ATP for 1 h. Subsequently the ghosts were washed three times with 50 mM Tris-HCl (pH 8.0; 10 mM NaF) by centrifugation (12000 × g; 5 min; 2 °C). Then, aliquots of $5 \cdot 10^6$ ghosts were taken and suspended in 100 μl 50 mM Tris-HCl (pH 8.0; 1 mM $MgCl_2$) containing poly(A) and/or ATP; the specific radioactivity of [^{3}H]poly(A) was adjusted to 0.8 Ci/mmol (with respect to phosphate content). After incubation for 10 min at 20 °C, 75-μl aliquots were layered over a 100-μl 30% (w/v) sucrose cushion and centrifuged in an A 100/30 rotor of a Beckman airfuge as described [30]. The radioactivity of the pelleted material was determined. Parallel assays were treated with 0.8 M urea (10 min; 10 °C); the ghost material was obtained by centrifugation (20000 × g; 10 min; 10 °C) and counted for radioactivity. Mean results of four independent experiments are given; the S.D. is less than 10%.

Assay	pmol ^{32}P incorporated per 10^6 ghosts	Treatment with	pmol [^{3}H]poly(A) bound per 10^6 ghosts	pmol ^{32}P incorporated per 10^6 ghosts	
				before urea treatment	after urea treatment
1	14.3	–	–	14.3	14.3
2	14.3	0.2 nmol poly(A)	–	13.9	13.8
3	14.3	0.2 nmol [^{3}H]poly(A)	7.4	–	–
4	14.3	0.2 nmol [^{3}H]poly(A) plus 5 μM ATP	5.3	–	–
5	14.3	0.2 nmol poly(A) plus 5 μM ATP	–	13.8	13.8

phosphatase amounted approximately to 25% of that determined for protein phosphokinase. However, the activity of nucleoside triphosphatase exceeded by far (approximately by 5000-fold in the oviduct and by 500-fold in the liver) those of the kinase and of the phosphatase.

Since the work of Agutter et al. [2] it is well established that ATP stimulates the release of bound poly(A) from the pore-laminae. However, no direct data are available which support the assumption that P_i is liberated from the proposed 'phosphorylated carrier' [1]. In a direct approach, we phosphorylated nuclear ghosts with [^{32}P]ATP in the standard kinase assay. Unbound ATP was removed by three successive washes. Subsequently the amount of labelled phosphate, bound to the envelope, was determined after incubation in the presence of 0 or 0.2 nmol poly(A) per assay (Table IV; assay 1 and 2). In the presence of poly(A), less than 5% of the originally incorporated phosphate was found to be released from nuclear ghosts. Considering the fact that the standard deviations of the data vary between 7 and 10%, we conclude that no phosphate release occurred during poly(A) binding to nuclear envelopes. To rule out the possibility that the labelled phosphate which was found to be associated with the ghosts, is due to residual amounts of [^{32}P]ATP, the ghosts were treated with 0.8 M urea. After this procedure no significant change of the amount of incorporated ^{32}P was observed. In an additional control experiment (Table IV; assay 3), it was demonstrated that, under the experimental conditions used, poly(A) was indeed bound to the ghosts. Addition of ATP to the incubation mixture containing poly(A) resulted in a reduction of the binding of the polymer to the ghosts by almost 30% (Table IV, assay 4). This finding confirms earlier observations [10]. In parallel experiments (Table IV; assay 5) it was determined that the amount of dephosphorylation of nuclear envelopes during the incubation with poly(A) and ATP was negligible. Taken together, we have no indication that a release of phosphate from nuclear envelope occurs after binding of poly(A) and/or ATP to this structure.

As one consequence of the ATP-mediated release of poly(A) from nuclear envelopes, a binding of ATP to the phosphorylated 'carrier' for poly(A) might be assumed. To test this hypothesis, pore-laminae were incubated with [^{3}H]ATP (Table V). After a 5-min incubation period the bound nucleotides were quantitatively determined. In the absence of poly(A), 23.9 pmol of ATP were measured to be bound to nuclear envelopes, isolated from $5 \cdot 10^6$ ghosts. In the presence of poly(A), the amount of bound ATP increased to 41.7 pmol. ADP was determined to be bound to a smaller extent, while only very little pore-laminae bound AMP could be identified. The latter finding is in

TABLE V

BINDING OF ATP TO PORE-LAMINAE

Pore-laminae were isolated from $5 \cdot 10^6$ nuclear ghosts from liver of mature animals. They were phosphorylated in the standard kinase assay for 60 min with unlabelled ATP. After three washes with 25 mM Tris-HCl buffer (pH 8.0; 150 mM NaCl, 10 mM NaF and 10 mM *O*-phospho-DL-serine), the resulting pore-laminae preparation was incubated (5 min; 20 °C) in a 1-ml assay in the presence of 1 μM [^{3}H]ATP (in 25 mM Tris-HCl (pH 8.0); 1 mM 2-mercaptoethanol, 2.5 mM $MgCl_2$, 150 mM NaCl, 10 mM NaF and 10 mM *O*-phospho-DL-serine). To assay 2, 100 μM poly(A) (with respect to phosphate content) was added. Subsequently the pore-laminae were washed five times by centrifugation (100000 × *g*; 10 min; 5 °C) until no radioactivity in the supernatant could be detected. Then the pore-laminae were treated with 1 ml of 0.8 M urea (10 min; 20 °C). After centrifugation, the released ATP, ADP and AMP were quantitatively determined. In a parallel series of experiments the incorporation of ^{32}P into $5 \cdot 10^6$ ghosts during a 60-min incubation period was determined using the standard kinase assay. Values are means of four independent determinations; the S.D. was less than 10%.

Assay	Incubation components	Nucleotides bound to pore-laminae (pmol/assay)			pmol of P_i incorporated into pore-laminae equivalents (based on $5 \cdot 10^6$ ghosts)
		ATP	ADP	AMP	
1	ATP	23.9	11.2	0.7	62.5
2	ATP plus poly(A)	41.7	13.6	0.9	62.5

accordance with the determination of Agutter [1], who found that the nucleoside triphosphatase product ADP is recycled to ATP via a myokinase-like reaction.

A stoichiometric comparison between the amount of ATP, non-covalently linked to pore-laminae, and the amount of phosphate, incorporated into this structure, revealed a 1 : 2.6 ratio in the absence and a 1 : 1.5 ratio in the presence of poly(A) (Table V). Because the yield achieved by the applied isolation procedure of pore-laminae from the nuclear ghosts was approx. 80%, a nearly 1 : 1 molar ratio between ATP binding and protein phosphorylation can be calculated.

Discussion

In the present report, the role of protein phosphokinase and protein phosphatase during the nuclear envelope nucleoside triphosphatase reaction was described. Under optimal reaction conditions, worked out by McDonald and Agutter [10] and slightly modified by us, linear time kinetics for both kinase and phosphatase up to 20 min were obtained. The nucleoside triphosphatase reaction proceeds linearly even for 30 min [2,7]. Being aware that the activities of enzymes which are associated with membrane structures are often multifactorially influenced, we performed in a first approach a comparison of the specific activities of the three enzymes to clarify if the kinase and the phosphatase constitute together the nucleoside triphosphatase as proposed [1]. In the nuclear ghost system of quails studied in the present contribution, the specific activities of the nuclear envelope-associated kinase and phosphatase differed only within a limited range; the kinase activity was approximately 3-fold higher than the phosphatase activity. However, the nucleoside triphosphatase activity exceeded 500-fold (liver system) or even 5000-fold (oviduct system) the activities of protein kinase and phosphatase (Table III). An equally large difference can be calculated from literature data [2,10,23,24].

Based on these enzymic data, we propose a new model to explain the nucleoside triphosphatase-

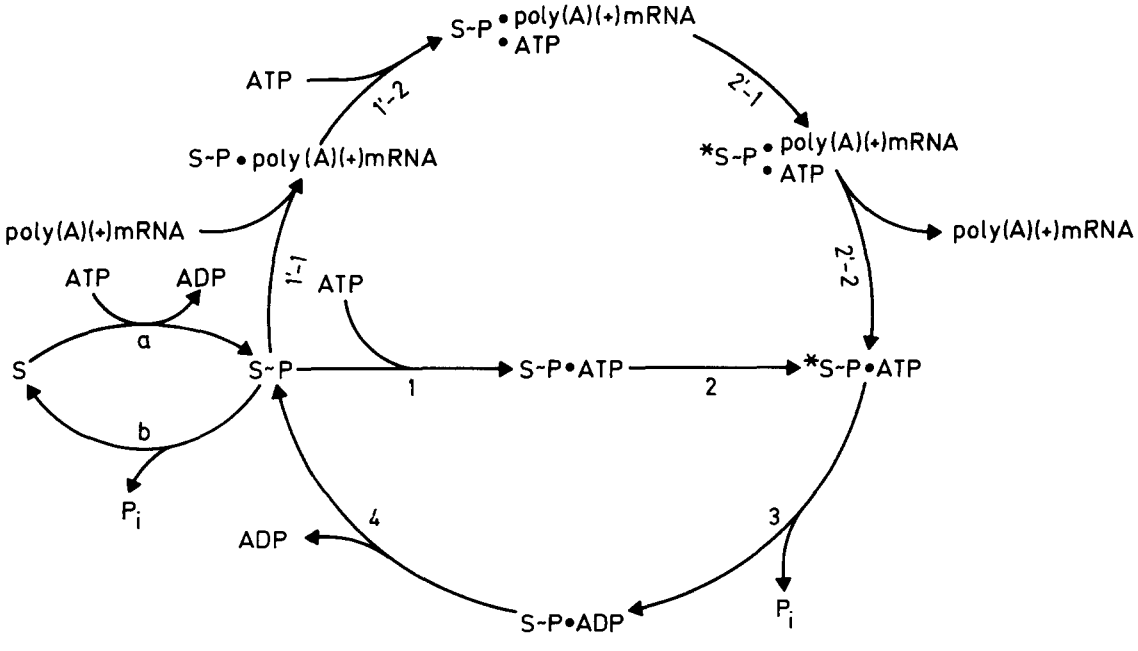

Fig. 5. Scheme for energy transduction by nuclear envelope nucleoside triphosphatase. The two 'carrier' conformations are depicted as S ~ P and *S ~ P. See text for further description. This scheme implies that the poly(A) binding site is identical with one of the polypeptides, involved in nucleoside triphosphatase reaction.

mediated transport of poly(A) (+)mRNA (Fig. 5). It is a modification of the general energy-transduction scheme introduced by Hill [25]. Under the assumption that the polypeptides involved in the nucleoside triphosphatase reaction are identical with the poly(A) binding site (a supposition which is not yet experimentally proven). ATP binds to the phosphorylated 'carrier', S ~ P, a reaction which can be deduced from the data in Table V (step 1). According to Hill, ATP binding results in a conformational change of S ~ P · ATP to *S ~ P · ATP (step 2); *S ~ P · ATP is a better enzyme for ATP hydrolysis than S ~ P · ATP (step 3). Splitting of phosphate from ATP (step 3) results in a conformational change from *S ~ P to S ~ P. According to Hill and supported by the presented experimental data (Table V), ADP has a low affinity to S ~ P and dissociates from the 'carrier' (step 4). The overall reaction velocity of the nucleoside triphosphatase cycle is accelerated if the system is coupled with the molecule [poly(A) (+)mRNA] to be transported. According to experimental data [10,26], poly(A) (+)mRNA binds preferentially to S ~ P rather than to S (step 1'-1). In step 1'-2, ATP binds to the S ~ P · poly(A) (+)mRNA complex which leads to a conformational change of the 'carrier' from S ~ P to *S ~ P (step 2'-1). In this complex, ATP causes the release of poly(A) (+)mRNA (step 2'-2), a reaction which is experimentally supported (Ref. 10; see also Table IV). This transition is analogous to the ATP-induced change of the myosin conformation [27]. In the proposed model for nucleoside triphosphatase-mediated mRNA transport, no dephosphorylation of the 'carrier' occurs (Table IV). This means that, in contrast to the model described earlier [1], phosphorylation (step a) and dephosphorylation (step b) of the 'carrier' are distinct reactions which proceed independently from the nucleoside triphosphatase cycle. Phosphorylation and dephosphorylation of the 'carrier' modulate only its affinity to poly(A)(+)mRNA and ATP as shown earlier [10,26]. The assumption that the phosphorylation/dephosphorylation cycle proceeds separately from the nucleoside triphosphatase cycle is supported additionally by the following finding. In the presence of 4 M urea nuclear pore complexes which are thought to contain the nucleoside triphosphatase [3] are extracted from nuclear envelopes [28], while the 'phosphorylated carrier' is not (unpublished results).

The experiments summarized here show that proteins in the nuclear envelope with M_r of 106000 and 64000 are phosphorylated during the protein kinase reaction. Further studies are needed to clarify if these proteins are distinct acceptors of, perhaps two different protein kinases [10,29] or, if they result from a preparation-dependent artifact due to the formation of disulfide bonds [30].

Confirming the data of McDonald and Agutter [10], it is shown that poly(A) stimulates protein phosphatase and inhibits protein kinase. This would mean that poly(A) is a regulatory effector for both nuclear envelope protein phosphorylation/dephosphorylation and nucleoside triphosphatase cycle [7,10,11].

Acknowledgements

The authors wish to thank Dr. P.S. Agutter (Napier College, Edinburgh) for encouragement and for helpful discussions. This research was supported by a grant from the Deutsche Forschungsgemeinschaft (Mu 348/7-4). We are indebted to Ms. R. Steffen for technical assistance.

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