Jpn. J. Cancer Res. 80, 1206–1211, December 1989

# Enhanced ATPase Activity in Liver Cell Nuclei Induced by Administration of Mitomycin C to Rats

Akira Wakizaka, Hirokazu Imai,[1] Namiko Aiba and Eiji Okuhara

*Department of Biochemistry, Akita University School of Medicine, 1-1-1 Hondoh, Akita 010*

**Intraperitoneal administration of mitomycin C (40 μg/100 g body weight) to male Wistar rats increased the ATPase activity in hypotonic extracts of liver cell nuclei for 4 days after injection. Partially purified ATPase, obtained by the DEAE-cellulose column chromatography of these extracts, showed a 14 times higher specific activity than that found in normal rat liver nuclei. The enzymatic activity was strongly enhanced by the addition of polynucleotides, especially poly A and poly I, to the assay mixture, but was inhibited by GTP, a chelating agent, heparin and thiol-group inhibitors. Quercetin and oligomycin were less effective, and ouabain showed no inhibitory effect. $Mg^{2+}$ ions were essential, but neither $Ca^{2+}$, $Na^{+}$ nor $K^{+}$ ions were required for the manifestation of the activity. These characteristic properties of the enzyme are similar to those of a nucleoside triphosphatase found in the nuclear matrix and envelope, suggesting that some energy-providing mechanisms involved in the repair processes of DNA damage or cellular injury are induced by mitomycin C administration.**

Key words: ATPase — Cell nucleus — Mitomycin C — Rat —Liver

Several characteristic species of ATPases or nucleoside triphosphatases (NTPases) have been found in eukaryotic cell nuclei as well as in other organisms.[1-13] The NTPase embedded in intact nuclear envelopes is markedly activated by synthetic poly A or the 3′-poly A tail of mRNA. It has also been demonstrated that such nuclear enzymes provide the energy for nucleocytoplasmic mRNA translocation.[1-9] Clawson *et al.*[1,3,4] showed that the enzymatic activity in nuclear envelopes was increased by the administration of various carcinogens to rats. In addition, they suggested that this phenomenon could play a role during the initiating phase of carcinogenesis.[4]

In a previous report, we observed an increase of DNA-dependent ATPase activity both in the cytoplasm and in the nucleus of mitomycin C (MMC)[2] administered rat liver cells.[14] Here, we describe the results of our further studies on extractable, nuclear ATPase.

## MATERIALS AND METHODS

**Chemicals** Polyethyleneimine (PEI)-cellulose pre-coated sheets for thin-layer chromatography were obtained from Macherey-Nagel, West Germany. [2,8-$^3$H]Adenosine 5′-triphosphate ([$^3$H]ATP) was obtained from NEN Research Products, Boston, Mass. Mitomycin C was kindly donated by Dr. T. Kato, Department of Urology, Akita University School of Medicine. Oligomycin was from Sigma Chemical Co., St. Louis, Mo. Poly A, poly I, poly U, and poly C were obtained from Yamasa Shoyu Co., Choshi, Chiba. Cyclohexanediaminetetraacetic acid and N-ethylmaleimide were purchased from Wako Pure Chemical Ltd., Osaka. *p*-Hydroxymercuribenzoate and phenylarsine oxide were from Aldrich Chemical Co., Milwaukee, WI. Phenylmethylsulfonyl fluoride (PMSF) was from Sigma Chemical Co. Ouabain was from E. Merck, Darmstadt, West Germany. Quercetin was from Funakoshi Chemical Co., Tokyo, and heparin sodium was from Takeda Chemical Industries Ltd., Osaka. DNA was prepared from salmon sperm as described previously.[15,16]

**Assay conditions for ATPase** Nuclear ATPase activity was measured by a modification of the method of Weinstock *et al.*[17] A 25 μl reaction mixture, containing 50 m*M* Tris-HCl buffer (pH 7.5), 0.1 m*M* $MgCl_2$, 1 m*M* DTT, 100 μg/ml bovine serum albumin, 100 μ*M* [$^3$H]-ATP (0.5 μCi) and an appropriate volume of the nuclear extract or purified enzyme fraction which had been dialyzed against 50 m*M* Tris-HCl buffer (pH 7.5) at 4°C for 2 to 3 h before the assay, was incubated at 37°C for 30 min. The reaction was terminated by the addition of 15 μl of a solution containing 25 m*M* EDTA and 3 m*M* ATP, ADP and AMP. Between 5 and 20 μl of the resulting mixture was applied to a PEI-cellulose thin-layer chromatography plate, which had been washed prior to use with a solvent mixture of 0.5 *M* LiCl and 1 *M* formic acid and dried in the air. The plate was developed in the same solvent system. Spots corresponding to ATP, ADP and AMP, which were detected under ultraviolet light, were cut out from the plate and their radioactivities were

[1] Present address: Department of Medicine, Akita University School of Medicine, 1-1-1 Hondoh, Akita 010.

[2] Abbreviations used are: MMC, mitomycin C; PEI, polyethyleneimine; ENP, extractable nuclear protein; LBP, loosely bound nuclear protein; PMSF, phenylmethylsulfonyl fluoride; DTT, dithiothreitol; dsDNA, double-stranded DNA; ssDNA, single-stranded DNA.

measured in a liquid scintillation counter. The rate of ATP hydrolysis was expressed as the ratio of the radioactivity counts of either ADP or ADP+AMP to the total counts of the three nucleotides.

**Extraction of nuclear ATPase** Male Wistar rats, obtained from Shizuoka Agricultural Cooperative Association for Laboratory Animals, Hamamatsu, Shizuoka, and weighing 250–300 g, were used throughout the experiment. Each animal was injected intraperitoneally with MMC at a rate of 40 $\mu$g/100 g body weight. The liver was removed from animals killed at various intervals after injection, and homogenized in 8 volumes of 0.25 *M* sucrose in buffer A containing 50 m*M* Tris-HCl (pH 7.5), 5 m*M* $MgCl_2$, 25 m*M* KCl, 0.1 m*M* PMSF and 1 m*M* DTT. The homogenate was first passed through 4 layers of gauze, then subjected to centrifugation at 3,000*g* for 15 min. The resulting pellet was resuspended in 8 volumes of 2.3 *M* sucrose in buffer A by a brief homogenization, and then resubjected to centrifugation at 60,000*g* for 60 min. The purified nuclei thus obtained were homogenized in 10 m*M* Tris-HCl buffer (pH 7.6) containing 0.1 m*M* PMSF and 1 m*M* DTT at a rate of 0.65 ml/g liver tissue, stirred at 4°C for 30 min, and then again centrifuged at 25,400*g* for 30 min. The resulting supernatant, designated as the extractable nuclear protein (ENP) fraction, was immediately mixed with the same volume of ethylene glycol and stored below −20°C until use. Residual precipitates were further homogenized with the same volume of 0.35 *M* NaCl containing buffer A. The mixture was allowed to stand at 4°C for 30 min with constant mixing, and was then subjected to centrifugation at 25,400*g* for 30 min. The extracts thus obtained was designated as the loosely-bound protein (LBP) fraction and stored in the same manner as above.

**Fractionation of nuclear ATPase** A part of the ENP or LBP fraction was mixed with 3 volumes of 50 m*M* Tris-HCl buffer (pH 7.6) containing 20% ethylene glycol, 0.1 m*M* PMSF and 1 m*M* DTT. The mixture was applied to a column of DEAE-cellulose, usually at a rate of 1 ml/0.1 ml of the ion exchange cellulose. The column was washed with 3–5 column volumes of the buffer, and the sample was then eluted stepwise with NaCl concentrations of 0.05, 0.15, 0.3, 0.5 and 1.0 *M* in the buffer.

## RESULTS

**Enhanced activity of ATP hydrolysis in rat liver nuclear extracts after MMC treatment** Liver cell nuclei were isolated from male Wistar rats at various intervals after MMC injection, and then successively extracted with either 10 m*M* Tris buffer (ENP) or the same buffer containing 0.35 *M* NaCl (LBP). Extracts were obtained separately from each of 5 rats, killed at intervals. The activity of ATP hydrolysis in the ENP and LBP fractions of each rat was then assayed. As shown in Fig. 1, MMC treatment at a dose of 40 $\mu$g/100 g body weight increased the activity of ATP hydrolysis in nuclear extracts one day after the injection. However, the administration of 1/10 amount of MMC, 4 $\mu$g/100 g body weight, did not increase the activity in the two fractions (data not shown). The activity in Fig. 1 is expressed as percent of radioactivity found in ADP and AMP molecules produced after the incubation, because some ATP was hydrolyzed to AMP, indicating that these nuclear extracts contained not only ATPase but also some other enzymes which could cleave ATP and/or ADP to AMP. The increases in the activity of both ENP and LBP fractions were statistically significant when compared with the control level (day 0). The activity of the ENP fraction continued at a high level for about 4 days, but gradually decreased to below the control level by the 6th day after injection. As shown in Fig. 1, the enhanced activity of the LBP fraction returned to the control level on the second day.

**Fractionation of nuclear ATPase** The ENP and LBP fractions obtained from the liver were subjected to DEAE-cellulose column chromatography in order to separate the nuclear ATPase from the other hydrolytic enzymes. The ENP and LBP fractions obtained from

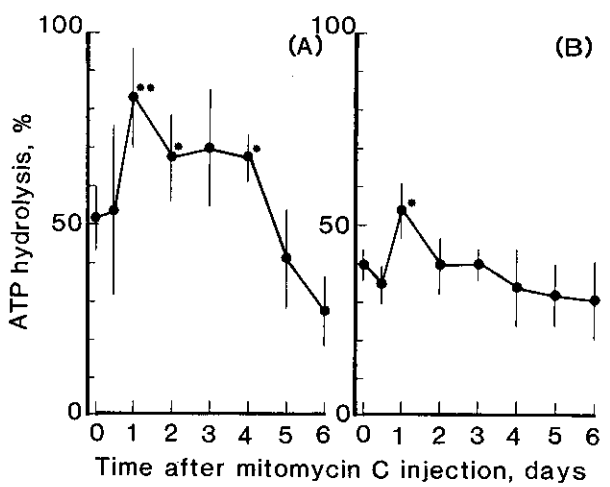


Fig. 1. Changes in the rate of ATP hydrolysis in rat liver nuclear extracts after mitomycin C administration. Aliquots of 10 $\mu$l of hypotonic extracts (A, ENP fraction) of the rat liver nuclei, or 0.35 *M* NaCl extracts of the residues (B, LBP fraction) were incubated with 0.1 m*M* [$^3$H]ATP in a 50 $\mu$l reaction mixture as described in "Materials and Methods." The rate of ATP hydrolysis is expressed as the sum of ADP and AMP ($\mu M$) formed during the incubation. The results are the means±SD of 5 rats. **, * Significantly different from the control (day 0) ($P<0.01$ and $P<0.05$, respectively) by Student's *t* test.

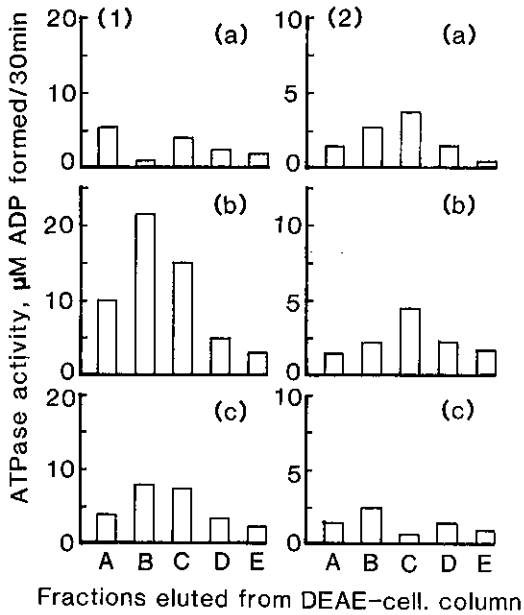


Fig. 2. Chromatographic separation of rat liver nuclear ATPase. Aliquots of 5.0 ml of combined ENP (1) or LBP (2) fractions from the rat liver before (a), and one day (b) or 5 days after (c) MMC administration were applied to a DEAE-cellulose column (1.2 cm ID × 5 cm). The column was successively eluted with 15 ml of 0.05 *M* (A), 0.15 *M* (B), 0.3 *M* (C), 0.5 *M* (D), and 1.0 *M* (E) NaCl. A 10 μl aliquot of each eluate was assayed for ATPase activity, which is expressed as % ADP formed.

each group of rats killed on the same day after MMC injection were applied separately to the column. The column was eluted stepwise with increasing NaCl concentrations in the Tris buffer. Fig. 2 shows that in the ENP fraction obtained one day after MMC treatment, the 0.15 and 0.3 *M* NaCl column eluates exhibited increased ATPase activities. In the LBP fraction, however, although a high activity of ATPase was observed in the 0.3 *M* NaCl eluate, the increment was not as distinct as that found in the 0.15 *M* NaCl eluate of the ENP fraction. With this chromatographic separation, the activity for hydrolysis of ATP to AMP during the incubation was greatly reduced to 1–2% of the sum of radioactivity found in the three nucleotide spots after thin layer chromatography. The protein concentration in the 0.15 *M* NaCl eluate of the ENP fraction was increased approximately three-fold over that of the control one day after treatment, whereas the ATPase activity in the same fraction was increased 44-fold by the treatment. The specific activity of the enzyme in the fraction obtained from treated rats was, therefore, 14-fold higher than that obtained from non-treated rats.

Table I. Enhancement of Nuclear ATPase Activity by the Addition of Polynucleotides

| Addition | ATPase activity[a] | |
|---|---|---|
| | Fraction eluted with 0.15 *M* NaCl | Fraction eluted with 0.3 *M* NaCl |
| None | 5.45±0.01 | 2.01±0.12 |
| poly A | 26.2±0.78 | 12.4±1.41 |
| poly I | 27.9±4.10 | 8.95±0.45 |
| poly C | 18.5±3.00 | 4.36±1.03 |
| poly U | 15.8±0.70 | 6.44±0.74 |
| dsDNA | 11.0±0.77 | 3.63±0.89 |
| ssDNA | 10.2±0.80 | 2.30±0.14 |

*a*) Results represent the mean±SD of three experiments. ATPase activity was measured with 0.5 μg protein from either the 0.15 *M* or 0.3 *M* NaCl fraction of ENP eluted from a DEAE-cellulose column. The reaction mixtures contained 0.1 mg/ml of various polynucleotides.

**Effect of polynucleotides on the enzyme activity** The ATPase activity in these nuclear extracts was greatly enhanced by the addition of polynucleotides to the assay mixture. Table I shows the effects of various polynucleotides on the ATPase activity of the 0.15 and 0.3 *M* NaCl eluates from the ENP fraction one day after the MMC treatment. By addition of poly A, the activity in both the 0.15 and 0.3 *M* NaCl eluates was increased to 5 to 6 times the control level. Poly I showed the same effectiveness as poly A on ATPase activity in the 0.15 *M* NaCl fraction, but somewhat less in the 0.3 *M* fraction. The pyrimidine polyribonucleotides, poly C and poly U, only moderately increased the ATPase activity. The enzymatic activity in the 0.15 *M* NaCl eluate was doubled by the addition of either dsDNA on ssDNA but their effects were low in the 0.3 *M* NaCl eluate. The 0.15 and 0.3 *M* NaCl eluates from the ENP fraction extracted from the normal rat liver nuclei also showed stimulatory effects on ATPase.

**Effect of chemical agents on ATPase activity** As shown in Table II, [$^3$H]ADP formation was considerably reduced by addition of non-radioactive ATP or GTP to the incubation mixture. This indicates that both nucleotides acted as competitive inhibitors of the enzyme, although GTP was less effective than ATP. This result indicates that the enzyme has a wide range of substrate specificities. The addition of 0.1 m*M* $MgCl_2$ to the assay mixture increased the ATPase activity to 158% of that without $Mg^{2+}$ ions, while concentrations over 1 m*M* rather decreased the activity (data not shown). The addition of $Ca^{2+}$, $K^+$ or $Na^+$ ions at concentrations of 0.01 to 100 m*M* did not increase the activity. A chelating agent, *t*-1,2-cyclohexanediaminetetraacetic acid, inhibited the activity almost completely at a concentration

Table II. Effects of Various Chemical Agents on the Nuclear ATPase Activity in the ENP Fraction after DEAE-Cellulose Column Chromatography

| Addition | m*M* | ATPase activity[a] | |
|---|---|---|---|
| | | Fraction eluted with 0.15 *M* NaCl | Fraction eluted with 0.3 *M* NaCl |
| None | – | 25.9±0.91 | 12.5±1.48 |
| ATP | 10 | 2.06±0.13 | 1.09±0.05 |
| | 1 | 3.34±1.03 | 2.56±0.08 |
| GTP | 10 | 3.62±1.46 | 1.02±0.15 |
| | 1 | 4.89±0.19 | 4.63±0.62 |
| N-Ethylmaleimide | 25 | 4.75±0.38 | 2.43±0.12 |
| | 5 | 5.94±1.10 | 4.80±1.38 |
| Phenylarsine oxide | 2.5 | 7.49±0.02 | 5.41±0.49 |
| | 0.5 | 9.18±0.06 | 5.56±0.06 |
| *p*-Hydroxymercuribenzoate | 5 | 3.20±0.19 | 1.30±0.02 |
| | 1 | 3.53±0.08 | 1.41±0.12 |
| Heparin | 0.1[b] | 4.78±0.09 | 1.72±0.16 |
| Quercetin | 0.25 | 17.2±0.98 | 9.23±1.16 |
| | 0.05 | 19.5±2.20 | 10.2±0.07 |
| Oligomycin | 25 | 19.5±0.77 | 10.0±0.44 |
| | 5 | 16.8±1.55 | 11.2±0.14 |

*a*) Results represent the mean±SD of three or more experiments.
*b*) mg/ml.
The reaction mixtures contained 0.1 mg/ml poly A and the same amount of the enzyme as used in the experiment shown in Table I.

of 10 m*M*. Addition of ouabain, a potent inhibitor of $Na^+$- and $K^+$-dependent ATPase, did not decrease the activity of this nuclear ATPase at concentrations of 0.1 and 1 m*M*. The addition of thiol group inhibitors, N-ethylmaleimide, phenylarsine oxide, and *p*-hydroxymercuribenzoate, effectively inhibited the nuclear ATPase. Heparin greatly inhibited the activity, while quercetin and oligomycin only inhibited the activity to some extent.

## DISCUSSION

It has been shown that nuclear ATPase or NTPase is an essential component in the process of mRNA translocation from the nucleus to the cytosolic compartment. Two different types of enzyme, NTPases I and II, were extracted from rat hepatic cell nuclei with a hypotonic solution by Blanchard and Richardson.[18] A 174 kDa ATPase/dATPase was found in the *Drosophila* nuclear matrix-pore complex-lamina fraction and in the rat liver nuclear pore complex by Berrios *et al.*[13, 19] An ATPase was solubilized from the nuclear envelope by Smith and Wells,[20] and another NTPase isolated from the rat liver nuclear envelope and matrix by Schroeder *et al.*[21] All of these enzymes have analogous functions but are different in several properties, such as polynucleotide dependency, substrate specificity, and the effect of chemical agents. Addition of poly A to the assay mixture increased the activity of the envelope-associated enzyme described by Schroeder *et al.*,[21] while poly U and poly C were ineffective.[21] In contrast, the activity of NTPase I[18] was enhanced by polynucleotides in the order of poly U>poly A>poly I>poly C, while NTPase II activity was enhanced in the order of poly I>poly A>poly C>poly U. The ATPase activity found in the present study, was equally increased by the addition of poly A or poly I, while poly U and poly C were less effective. This appears to be similar to the activity of NTPase II, as reported by Blanchard and Richardson.[18]

An NTPase in the rat liver nuclear envelope catalyzes the hydrolysis of ATP and GTP,[9, 21] but the matrix enzyme does not utilize GTP.[3] NTPase I in hypotonic extracts of rat liver nuclei acts only on ATP and dATP, while NTPase II in the same extract hydrolyzes four ribonucleoside triphosphates and dATP, to different extents.[18] The extractable nuclear ATPase reported in the present study seems to specifically catalyze ATP hydrolysis and, to a slightly lesser extent, the hydrolysis of GTP. This mode of action is also similar to the above-mentioned nuclear NTPase II reported by Blanchard and Richardson,[18] and taken together with the response to specific polynucleotides, strongly suggests that the nuclear extractable ATPase corresponds to NTPase II.

One of the most characteristic properties of the extractable nuclear ATPase in the present study was that the activity is completely inhibited by the addition of heparin. The thiol group reagents, N-ethylmaleimide, *p*-hydroxymercuribenzoate, and phenylarsine oxide, acted as effective inhibitors, as in the case of the nuclear envelope NTPase.[21] However, the effects of quercetin and oligomycin on the enzyme were very different from the effects on the envelope enzyme, which was strongly inhibited by these agents.[9, 21] Such observations imply that the ATPase present in the nuclear envelope is different from that in the soluble fraction. No report has described the effects of these chemical agents on NTPase I and II activities in hypotonic nuclear extracts.

The extractable ATPase from the whole cell nuclei and the ATPase bound to the nuclear envelope or matrix differ in several respects, as mentioned above. However, as far as the enhancement of activity by polyribonucleotides is concerned, these two ATPases are fundamentally similar. This would imply that these nuclear ATPases or NTPases have similar functions in the cell nucleus.

In the present study, ATPase or NTPase activity was widely observed in several nuclear fractions when extracted with different salt concentrations and separated using anion-exchange chromatography. This might be partly caused by the degradation of the enzyme during the extraction process. But another explanation is possible; that is, nuclei contain several ATPases with similar function and slightly different chemical structures. These ATPases may interconvert through processes such as phosphorylation and dephosphorylation, which may provide a mechanism to regulate the enzyme activity. In rats not treated with MMC, the ATPase activity in the liver was found mainly in the 0.3 *M* NaCl eluate from the LBP fraction. The dose-response relation of MMC was not examined in the present study, because the purpose of the present experiment was focused on the characterization of the ATPase which is extractable from nuclei. However, as already mentioned, a single injection of MMC at a rate of 4 $\mu$g/100 g body weight, which is a regular daily dose of the drug for cancer patients, did not cause any measurable increase of the enzymatic activity. This might indicate that a certain amount of DNA damage or cellular injury should be accumulated in order to manifest the increased nuclear ATPase activity. The administration of 40 $\mu$g/100 g body weight of MMC, 10 times larger than the ordinary dose, increased the ATPase activity in the ENP fraction, not in the LBP fraction, suggesting loss of affinity of the enzyme for the particulate material of nuclei. Further, the main ATPase activity was eluted from the column at a much lower NaCl concentration than in the case of nuclear extracts of normal control rats. These results indicate that the cell nucleus contains a variety of ATPases or NTPases with analogous functions, and MMC treatment resulted in decreases of their anionic nature and binding forces to the nuclear structural components, such as the envelope, matrix or chromatin, through some mechanism, possibly dephosphorylation of the enzyme. Such structural changes would appear to enhance the activity of the enzyme. However, the enhancement of the activity may be induced through a different kind of mechanism, such as an increase in biosynthesis of the enzyme for some unknown reason, followed by accumulation in nuclei. The latter possibility should be examined by the use of specific antibodies or by evaluating the effect of protein synthesis inhibitors. Such studies are in progress in our laboratory.

Even though the precise mechanism is still unknown, the involvement of the enzyme in RNA translocation has been demonstrated by several observations.[6, 11, 22] Furthermore, the increase in ATPase or NTPase activity by treatment with MMC and thioacetamide, as well as other carcinogens,[1, 3] suggests that nuclear ATPase or NTPase functions during the damage and repair processes of DNA and/or during subsequent cellular changes in metabolism.

*(Received July 8, 1989/Accepted November 1, 1989)*

REFERENCES

1) Clawson, G. A., Woo, C. H. and Smuckler, E. A. Independent responses of nucleoside triphosphatase and protein kinase in nuclear envelope following thioacetamide treatment. *Biochim. Biophys. Res. Commun.*, **95**, 1200–1204 (1980).
2) Purello, F., Vigneri, R., Clawson, G. A. and Goldfine, I. D. Insulin stimulation of nucleoside triphosphatase activity in isolated nuclear envelopes. *Science*, **216**, 1005–1007 (1982).
3) Clawson, G. A. and Smuckler, E. A. Increased nucleoside triphosphatase activity of rat liver nuclear matrix following low dose carcinogen intoxication. *Biochim. Biophys. Acta*, **108**, 1331–1339 (1982).
4) Clawson, G. A., Moody, D. E., Ferrell, L. D. and Smuckler, E. G. Increased nucleoside triphosphatase activity of rat liver nuclear envelope is associated with hepatocarcinogen exposure *Lab. Invest.*, **51**, 682–689 (1984).
5) Clawson, G. A., Friend, D. S. and Smuckler, E. A. Localization of nucleoside triphosphatase activity to the inner nuclear envelope and associated hepatochromatin. *Exp. Cell Res.*, **155**, 310–314 (1984).

6) Clawson, G. A., Button, J. and Smuckler, E. A. Photoaffinity labeling of a nuclear matrix nucleoside triphosphatase and its modulation in the acute-phase response. *Exp. Cell Res.*, **159**, 171–175 (1985).
7) Bernd, A., Schroeder, H. C., Zahn, R. K. and Mueller, W. E. G. Nuclear-envelope nucleoside triphosphatase: stimulation by poly(A)(+)mRNA and modulation by microtubule protein. *Akt. Gerontol.*, **13**, 119–121 (1983).
8) Kondor-Koch, C., Riedel, N., Valentin, R., Fasold, H. and Fischer, H. Characterization of an ATPase on the inside of rat-liver nuclear envelopes by affinity labeling. *Eur. J. Biochem.*, **127**, 285–289 (1982).
9) Agutter, P. S., McArdle, H. J. and McCaldin, B. Evidence for involvement of nuclear envelope nucleoside triphosphatase in nucleocytoplasmic translocation of ribonucleoprotein. *Nature*, **263**, 165–167 (1976).
10) Paoleti, E. and Moss, B. Two nucleic acid-dependent nucleoside triphosphate phosphorylases from vaccinia virus. *J. Biol. Chem.*, **249**, 3281–3286 (1974).
11) Baglia, F. A. and Maul, G. G. Nuclear ribonucleoprotein release and nucleoside triphosphatase activity are inhibited by antibodies directed against one nuclear matrix glycoprotein. *Proc. Natl. Acad. Sci. USA*, **80**, 2285–2289 (1983).
12) Chu, A. S. and Richardson, J. P. An RNA-dependent ATPase from *Chlamydomonas reinhardii*. *Biochim. Biophys. Acta*, **653**, 378–390 (1981).
13) Berrios, M., Blobel, G. and Fisher, P. A. Characterization of an ATPase/dATPase activity associated with *Drosophila* nuclear matrix-pore complex-lamina fraction. *J. Biol. Chem.*, **258**, 4548–4555 (1983).
14) Imai, H. The induction of Rec A-like proteins in the rat liver with mitomycin C (in Japanese). *Akita J. Med.*, **11**, 677–692 (1985).
15) Wakizaka, A. and Okuhara, E. Immunochemical studies on the correlation between conformational changes of DNA caused by ultraviolet irradiation and manifestation of antigenicity. *J. Biochem.*, **86**, 1469–1478 (1979).
16) Wakizaka, A. and Okuhara, E. Immunologically active lesions induced on double-stranded DNA with ultraviolet. *Photochem. Photobiol.*, **30**, 573–579 (1979).
17) Weinstock, G. M., McEntee, K. and Lehman, I. R. Hydrolysis of nucleoside triphosphate catalyzed by the recA protein of *Escherichia coli*. Characterization of ATP hydrolysis. *J. Biol. Chem.*, **256**, 8829–8834 (1981).
18) Blanchard, K. L. and Richardson, J. P. Two ribonucleic acid-dependent nucleoside triphosphatases from rat liver nuclei. *J. Biol. Chem.*, **258**, 14091–14097 (1983).
19) Berrios, M., Filson, A. J., Blobel, G. and Fisher, P. A. A 174-kilodalton ATPase/dATPase polypeptide and a glycoprotein of apparently identical molecular weight are common but distinct components of higher eukaryotic nuclear structural protein subfraction. *J. Biol. Chem.*, **258**, 13384–13390 (1983).
20) Smith, C. D. and Wells, W. W. Solubilization and reconstitution of a nuclear envelope-associated ATPase. *J. Biol. Chem.*, **259**, 11890–11894 (1984).
21) Schroeder, H. C., Rottmann, M., Bachmann, M. and Mueller, W. E. G. Purification and characterization of the major nucleoside triphosphatase from rat liver nuclear envelopes. *J. Biol. Chem.*, **261**, 663–668 (1986).
22) Schroeder, H. C., Nitzgen, D. E., Bernd, A., Krelec, B., Zahn, R. K., Gramzow, M. and Mueller, W. E. G. Inhibition of nuclear envelope nucleoside triphosphate-regulated nucleocytoplasmic messenger RNA translocation by 9-β-D-arabinofuranosyladenine 5′-triphosphate in rodent cells. *Cancer Res.*, **44**, 3812–3819 (1984).