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Identification of a wheat germ agglutinin-sensitive ATPase in yeast nuclei

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We have found that wheat germ agglutinin (WGA), a lectin that specifically binds to *N*-acetylglucosamine residues inhibits the in vitro transport of plasmid DNA, pJDB219, into yeast nuclei. Histochemical staining of the isolated nuclei with biotinylated WGA and streptavidin-biotinylated peroxidase complex revealed the presence of WGA-binding materials around the nuclear pore under an electron microscope. Using WGA-agarose column chromatography of yeast nuclear extracts, a novel Mg^{2+}-dependent ATPase was isolated. Its activity was highly sensitive to WGA and stimulated by Nonidet P-40 or phosphatidylserine. We suggest that the WGA-sensitive ATPase plays a role in yeast nuclear transport of DNA.

ATPase, nuclear; Nuclear transport; Wheat germ agglutinin-binding protein; *(Saccharomyces cerevisiae)*

1. INTRODUCTION

Eukaryotic cells have a nucleus surrounded by a double membrane. The transport of cellular materials between the cytoplasm and nucleus is believed to occur through a nuclear-pore complex which fuses the inner and outer nuclear membranes. The nuclear-pore complex is a large organelle composed of eight subunits with a mass estimated to be about 10^8 Da [1–3]. There is growing evidence that macromolecular traffic through the nuclear-pore complex is strictly regulated, and the regulatory mechanisms are important for the proliferation and differentiation of eukaryotic cells [4–6]. Little is known about the molecular structure and mechanism of the nuclear-pore complex. However, the presence of proteins bearing *O*-linked *N*-acetylglucosamine (GlcNAc) around the nuclear-pore complex of rat liver nuclei was recently reported [7–9].

To investigate the mechanisms of macromolecule transport through the nuclear-pore complex, we recently established an in vitro assay system for nuclear transport with isolated yeast nuclei and plasmid DNA (pJDB219, 7.2×10^6 Da) [10,11]. We found that nuclear ATPase and protein kinase activities are indispensable for this transport process [11]. In this paper, we describe our finding that wheat germ agglutinin (WGA), a lectin which specifically binds to GlcNAc residues, inhibits the transport of pJDB219 in vitro, and that a novel ATPase highly sensitive to WGA is present in the yeast nuclear extracts.

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Abbreviations: WGA, wheat germ agglutinin; GlcNAc, *N*-acetylglucosamine; Hepes, *N*-2-hydroxyethylpiperadine-*N'*-2-ethanesulfonic acid; NP-40, polyoxyethylele (9) *p-t*-octylphenyl ether (Nonidet P-40); DCCD, *N,N'*-dicyclohexylcarbodiimide

2. MATERIALS AND METHODS

2.1. *Yeast strain and purification of nuclei*

Cultivation of the yeast *Saccharomyces cerevisiae* A364A and purification of nuclei were done as described previously [12], ex-

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cept that cell breakage was done by a freeze and blast disrupting method using a Cryo-Clean Blaster CCB-100B (Taiyo Sanso Co. Ltd., Osaka, Japan). Employing this disruption method, we recovered about 2.5 times higher ATPase activity in the nuclear extract than obtained by our previous procedures.

2.2. *Assay of DNA uptake by the isolated nuclei*

The assay for plasmid DNA (pJDB219) uptake into isolated nuclei was described in our previous paper [11]. After the reaction at 28°C for 10 min, nuclei were separated from unincorporated pJDB219 by density gradient centrifugation. Nuclei recovered in the pellet were lysed in the sample buffer (1% (w/v) SDS, 8% (w/v) glycerol, 0.02% (w/v) bromophenol blue), and the DNAs in the samples were separated by gel electrophoresis and detected by ethidium bromide staining.

2.3. *Electron microscopy*

Nuclei were fixed with 3% paraformaldehyde in SMPBS (0.4 M sorbitol, 1 mM $MgCl_2$, 10 mM sodium phosphate, pH 7.5, and 0.15 M NaCl) at room temperature for 30 min, washed twice with SMPBS and incubated in SMPBS containing 25 μg/ml of biotinylated WGA (Vector Laboratories Inc., Burlingame, USA) at room temperature for 30 min. After washing twice with SMPBS, the nuclei were incubated with the streptavidin-biotinylated peroxidase complex (Amersham Japan, Tokyo, Japan) at room temperature for 30 min, washed twice with PBS (10 mM sodium phosphate, pH 7.5, and 0.15 M NaCl) and embedded in small agar blocks. Peroxidase reaction was carried out by soaking the agar blocks in PBS containing 0.5 mg/ml of 3,3'-diaminobenzidine tetrahydrochloride, 0.03% $CoCl_2$ and 0.02% H_2O_2 at room temperature for 1 min followed by 4 distilled water washes and post-fixation with 1% O_3O_4 at 2°C for 30 min. Dehydration, embedding into electron microscopic resin and preparation of ultrathin sections were as described previously [13]. The thin sections were observed under a Hitachi H-600A electron microscope (Hitachi Ltd, Tokyo, Japan) at 100 kV.

2.4. *Assay of ATPase activity*

ATPase activity was assayed in a reaction mixture composed of 20 mM Hepes-KOH, pH 7.0, 15 mM KCl, 10 mM $MgCl_2$, 5 mM dithiothreitol, 500 μg/ml bovine serum albumin, 0.05% (w/v) NP-40 and 1 mM [γ-^{32}P]ATP (ICN Biochemicals, Irvine, USA, 4.5 mCi/mmol) according to the method described by Hachmann et al. [14] at 28°C.

2.5. *Preparation of a nuclear extract and a WGA-agarose column chromatography*

2×10^{11} nuclei were incubated in 0.2% (w/v) Nonidet P-40 (NP-40, BDH Chemicals Ltd, Poole, England), 20 mM Tris-HCl, pH 7.0, 1 mM $MgCl_2$ and 5 mM β-mercaptoethanol at 2°C for 10 min, and centrifuged at 50 000 $\times$ g for 2.5 h. The nuclear extract obtained as a supernatant was then applied to a WGA-agarose column (Seikagaku Kogyo Co. Ltd, Tokyo, Japan, 2 ml) equilibrated with 20 mM Hepes-KOH, pH 7.0, 2 mM $MgCl_2$, 1 mM dithiothreitol, 100 mM KCl and 0.05% (w/v) NP-40 (buffer A). After washing the column with 5 bed vols each of buffer A containing 0.15 M NaCl and buffer A, WGA-binding materials were eluted with buffer A containing 0.5 M GlcNAc.

3. RESULTS AND DISCUSSION

The isolated yeast nuclei incorporated plasmid DNA, pJDB219, in an ATP-dependent manner. pJDB219 added to the reaction was detected in the nuclei within 10 min and was accompanied by a change of the DNA into a linear form as described previously (fig.1, lane 3) [11]. In the presence of 50 μg/ml WGA, plasmid DNA incorporation was inhibited (fig.1, lane 4). Addition of 0.1 M GlcNAc, a competitive inhibitor of WGA, prevented WGA repression of the incorporation (fig.1, lane 5). WGA did not seem to enhance nuclear DNase activity because the band of chromosomal DNA in gel lane 4 was identical to that in lanes 2 and 3. Thus, the yeast nucleus appears to possess WGA-binding components involved in macromolecule transport across the nuclear membrane.

The locations of WGA-binding materials in yeast nuclei were investigated by histochemical staining of the isolated nuclei with biotinylated

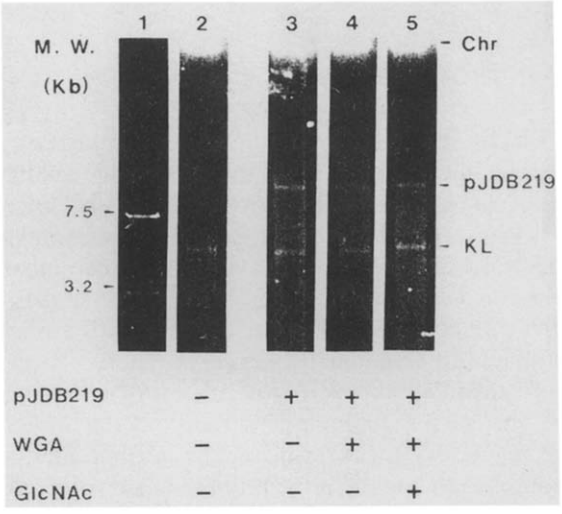

Fig.1. Effect of wheat germ agglutinin on DNA uptake activity of the isolated yeast nuclei. The isolated nuclei (1.5×10^8) were incubated with 0.5 μg of pJDB219 at 28°C for 10 min, in the absence (lane 3) or in the presence of 50 μg/ml of wheat germ agglutinin (WGA, lane 4) and WGA plus 0.1 M *N*-acetylglucosamine (lane 5). After the incubation, nuclei were recovered in the pellet of a density gradient centrifugation. DNAs in the nuclei were separated by agarose gel electrophoresis and detected by ethidium bromide staining. Detailed assay procedure is reported in our previous paper [11]. Lane 1, DNA size marker; lane 2, nuclei incubated without pJDB219; Chr, chromosomal DNA; KL, killer RNA.

WGA and the streptavidin-biotinylated peroxidase complex, under an electron microscope. As shown in fig.2 (panel B), electron dense deposits were observed around nuclear pores and on the intranuclear face of nuclear membrane near the pore structure of WGA-stained nuclei (indicated with arrows and arrowheads, respectively). The electron dense deposits were not observed in these regions of the nuclei without biotinylated-WGA binding (fig.2, panel A).

In order to identify the WGA-binding components in the yeast nucleus, we prepared a nuclear extract containing membrane constituents and separated the WGA-binding components by immobilized WGA-agarose column chromatography. Since nuclear ATPase and protein kinase are required for in vitro DNA-uptake by the nuclei, we surveyed these enzyme activities in the 0.5 M GlcNAc eluates of the column. ATPase activity but no protein kinase activity was detected in these fractions (fig.2). Pooled ATPase fractions were used for the experiments of table 1. The ATPase activity was repressed by the addition of WGA (25 μg/ml, fig.1 and table 1). Furthermore, this ATPase required Mg^{2+} and a detergent, NP-40 (0.05%), for full activity (table 1). In the absence of NP-40, the activity decreased to 40%, but was restored by the addition of phosphatidylserine (20 μg/ml, table 1). The data indicate that this nuclear ATPase is a membrane-associated enzyme.

To characterize this ATPase activity, we tested the effects of various ATPase inhibitors. As shown in table 1, the activity was resistant to all the inhibitors tested (NaN_3, quercetin, sodium vanadate, DCCD and ouabain). Therefore, the behaviour of this nuclear WGA-binding ATPase to these inhibitors differs from other known ATPases of yeast, such as those of the plasma membrane, mitochondria or vacuole [15].

This is the first report describing the presence of a WGA-sensitive ATPase in yeast nuclei. The precise location of this enzyme within the nucleus is unknown, however, our results strongly suggest the possibility that the ATPase we have found plays a role in the macromolecule transport system of

Table 1

Effects of various reagents on the nuclear WGA-binding ATPase

Reagent (conc.)		ATPase activity (P_i released, cpm/5 min)
None (complete)		2188 (100)
+ NaN	(10 mM)	2046 (94 ± 2)
+ Quercetin	(20 μg/ml)	2117 (96 ± 5)
+ Sodium vanadate	(2 mM)	2074 (92 ± 5)
+ DCCD	(1 mM)	2016 (91 ± 3)
	(1.5 mM)	2281 (102 ± 2)
+ EDTA	(1 mM)	403 (20 ± 2)
+ EGTA	(2 mM)	2044 (94 ± 2)
+ WGA	(25 μg/ml)	449 (21 ± 3)
− NP-40		875 (40 ± 2)
− NP-40 + phosphatidylserine	(20 μg/ml)	1599 (75 ± 3)

Numbers in parentheses in the rightmost column show the averaged values of 4 experiments in relative activities

Fig.2. Electron microscopic localization of WGA-binding materials in isolated nuclei. The indirect staining method using biotinylated WGA and streptavidin-biotinylated peroxidase complex was used to label the isolated nuclei (panel B). In panel A, the step of biotinylated-WGA binding was omitted. Open arrows indicate nuclear pores. Arrows and arrowheads in panel B show electron dense deposits on the nuclear pore and on the intranuclear face of nuclear membrane, respectively.

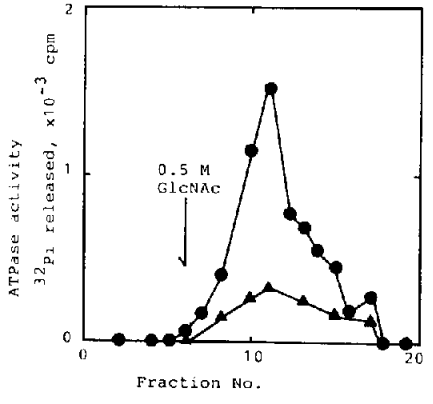

Fig.3. WGA-agarose column chromatography of the yeast nuclear extract. ATPase activity was assayed in the absence (●) or in the presence (▲) of 25 μg/ml WGA.

yeast nuclei. Further purification and characterization of the enzyme is in progress in our laboratory.

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