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Rapid Report

A 46 kDa NTPase common to rat liver nuclear envelope, mitochondria, plasma membrane, and endoplasmic reticulum

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A 46 kDa ATP binding polypeptide of the nuclear envelope, virtually identical to the nuclear envelope NTPase putatively involved in mRNA efflux [6], is present in all rat liver cell membranes. Its presence in nuclear envelope is not the result of cross contamination during isolation.

Considerable progress has been made towards the characterization of the major rat liver nuclear envelope NTPase [1–5]. This enzyme has been postulated to play a pivotal role in insulin stimulated mRNA efflux from the nucleus [6–8]. Commensurate with its putative role as a component of the energy generator for the nucleocytoplasmic translocation of mRNA, this enzyme is located in the nuclear envelope, probably in the pore complex [2].

Probing for rat liver ATP binding polypeptides using 8-azidoadenosine 5′-[γ-^{32}P]triphosphate (prepared enzymatically [9]), revealed the presence of an approximately 46 kDa polypeptide not only in the nuclear envelope but also in plasma membrane, and mitochondria (Fig. 1A, lanes 1–3). It does not undergo phosphorylation under the conditions used (Fig. 1B). It has the same isoelectric point (Fig. 1C) irrespective of membrane type in which it occurs, thus providing further evidence that the same 46 kDa protein is present in all the investigated membrane structures. Its molecular size is different from that of the β-subunit of the mitochondrial membrane ATPase [18] and of actin, from which it is well separated on SDS gel electrophoresis (results not shown).

To further characterize the 46 kDa polypeptide, the three membrane types were preincubated with the inhibitors quercetin, oubain and colchicine prior to the ATP binding test (Fig. 1D). Oubain (lane 3) has no effect, preincubation with quercetin results in a considerable decrease in ATP binding activity (lane 2). Colchicine is less effective than quercetin (lane 4). The ubiquitous presence of the 46 kDa polypeptide is not an artefact due to cross contamination, as demonstrated in Fig. 1D.

It has been suggested that the major ATP binding protein of the rat liver nuclear envelope is involved in the mechanism of insulin induced mRNA efflux from the nucleus [6–8]. This investigation identifies an ATP binding polypeptide identical to the abovementioned NTPase, i.e., the same molecular weight of 46 kDa (Fig. 1A), ATP binding (Fig. 1B, lanes 1–3), absence of phosphorylation (Fig. 1B, lanes 4–6), and inhibition characteristics (Fig. 1D) similar to those described previously [2].

The 46 kDa polypeptide, only a minor nuclear envelope component, is prominently labelled by N_3ATP, as described before [16]. The 46 kDa ATP binding polypeptides of the various membrane structures appear to be identical in molecular weight (Fig. 1A); isoelectric point (Fig. 1C); and inhibitor-sensitivity (Fig. 1D). We found this same polypeptide in membranes of a related (rat hepatoma HIIE), and unrelated line (murine erythroleukaemia cells), results not shown.

The presence of this ATP binding polypeptide in non-nuclear membranes suggests a more general role. In the nuclear membrane, by virtue of it's association

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Abbreviations: [γ-^{32}P]N_3ATP, 8-azidoadenosine 5′-[γ-^{32}P]triphosphate; βME, β-mercaptoethanol; TEMS, 40 mM Tris-HCl, 2 mM EDTA (pH 7.8), 10 mM magnesium chloride and 100 mM sodium chloride.

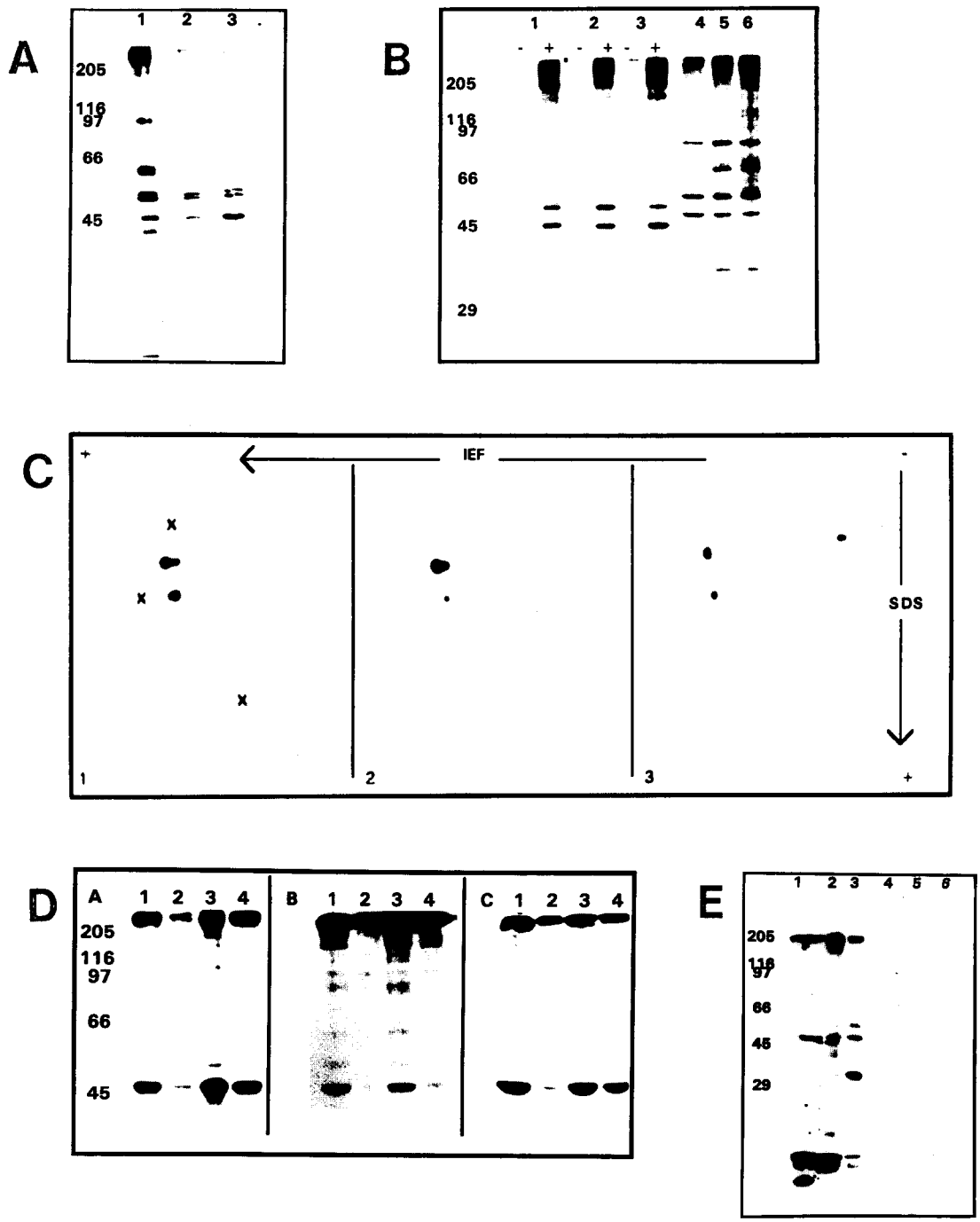

Fig. 1. (A) The N_3ATP binding profile of rat liver cellular substructures. Lane 1: Plasma membrane; lane 2: nuclear envelope; lane 3: mitochondria. Plasma membrane and mitochondria were prepared according to Aronson and Touster [14] and nuclear envelopes according to Bornens and Courvalin [10]. 100–200 μg of sample suspended in 250 μl of 0.25 M sucrose in TEMS, were incubated for 5 min with 5 μM [γ-^{32}P]N_3ATP (16 Ci/mmol) in the dark. The membranes were then pelleted, resuspended in fresh incubation medium, subjected to a photoflash unit with a 500V Xenon lamp [17]. After repelleting, the membranes were solubilised and analyzed by one- or two-dimensional SDS PAGE [15] and autoradiographed. (B) The phosphorylation and N_3ATP binding profile of rat liver nuclear envelopes isolated by different methods. Lanes 1–3: Nuclear envelopes were isolated from purified nuclei [13], according to methods described in Refs. 10–12, respectively. +, u.v. crosslinked, and −, control sample. Phosphorylation: lanes 4–6. Phosphorylation was measured by incubating 250 μg membranes for 5 min in 500 μl TMS, 0.25 M sucrose [γ-^{32}P]ATP (16 Ci/mmol) prior to PAGE. (C) Two-dimensional PAGE of N_3ATP labelled rat liver membrane fractions. Panel 1, nuclear envelope; Panel 2, plasma membrane; Panel 3, mitochondria. ×, marker proteins: bovine serum albumin (68 kDa); ovalbumin (45 kDa; p*I* 4.8); and carbonic anhydrase (29 kDa; p*I* 6.1). (D) The sensitivity of the rat liver 46 kDa N_3ATP binding protein to the inhibitors quercetin, oubain and colchicine. Panel A, nuclear envelope; Panel B, plasma membrane; Panel C, endoplasmic reticulum. For A–C: Lane 1, no inhibitor; lane 2, quercetin (33 μM); lane 3, oubain (1 mM); lane 4, colchicine (1 mM). 150 μg membranes were preincubated in 300 μl of TEMS for 5 min with quercetin (33 μM), oubain (1 mM), or colchicine (1 mM), prior to labelling. (E) Absence of cross contamination of the rat liver subcellular fractions investigated. $6 \cdot 10^6$ dpm of [γ-^{32}P]N_3ATP-labelled mitochondria, plasma membrane and endoplasmic reticulum (lanes 1–3) were added to three homogenates from which nuclear envelopes were isolated (lanes 4–6) and subjected to PAGE.

with other pertinent components, possibly in the nuclear pore complex, it may assist in mRNA efflux by providing energy for this process. In other membrane structures this ATP binding protein would obviously be involved in different, but possibly related processes, like the translocation of other macromolecules across membranes.

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