Purification and functional reconstitution of the human Wilson copper ATPase, ATP7B

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Abstract Wilson disease is a disorder of copper metabolism, due to inherited mutations in the Wilson copper ATPase gene *ATP7B*. To purify and study the function of the ATPase, the enzyme was truncated by five of the six metal binding domains and endowed with an N-terminal histidine-tag for affinity purification. This construct, Δ1–5WNDP, was able to functionally complement a yeast strain defective in its native copper ATPase CCC2. Δ1–5WNDP was purified by Ni-affinity chromatography and reconstituted into proteoliposomes. This allowed, for the first time, the functional study of the Wilson ATPase in a purified, reconstituted system.

Keywords: Copper homeostasis; Copper ATPase; Wilson disease; Purification; Overexpression; *Saccharomyces cerevisiae*

1. Introduction

Wilson disease is caused by mutations in the copper ATPase gene *ATP7B*. The disease is autosomal recessive and occurs at a frequency of about 1 in 30000. It is associated with a systemic overload of copper due to a lack of hepatic clearance of the trace element. Major clinical manifestations of Wilson disease are neurological impairment and hepatic cirrhosis. *ATP7B* encodes the Wilson disease protein (WNDP), a copper ATPase which is primarily expressed in the liver, but also in the brain, kidney, cornea and spleen. In other tissues, the homologous Menkes ATPase, ATP7A (MNKP), is expressed instead. Defects in the latter enzyme result in Menkes disease, a fatal defect in copper uptake by the organism (see [1–5]). It was shown by phosphorylation assays, yeast complementation assays, and transport studies with ^{64}Cu that WNDP and MNKP transport copper(I) and that this transport is ATP dependent [6–9].

WNDP is a 165 kDa protein consisting of 8 transmembrane helices and a long N-terminal tail with 6 metal binding modules, each containing a CxxC (in the one-letter amino acid code, which is used throughout) consensus copper binding motif. The enzyme is a member of the P-type ATPase family, which use the energy of ATP hydrolysis to transport cations across cell membranes. All P-type ATPases contain the characteristic signature sequence DKTGT. The aspartic acid of this motif is phosphorylated in the course of the reaction cycle, which led to the name P-type ATPases [10]. More than 150 members of the P-type ATPase family have been identified and divided into five subfamilies according to their sequence, cation specificity, membrane topology and the presence of different regulatory domains [11].

Copper ATPases, first discovered in bacteria in 1992 [12], marked the emergence of a new sub-family of P-type ATPases involved in the transport of heavy metal ions. They are commonly called P1-type ATPases or CPx-type ATPases [13,14]. CPx-type ATPases differ in several important aspects from non-heavy metal ATPases: (i) they feature one to six N-terminal metal binding sites (MBSs); (ii) following the MBSs, they have two additional transmembrane helices; (iii) they possess a conserved CPC or CPH motif in membrane helix six (hence the name CPx-type); (iv) they contain a conserved HP motif in the second cytoplasmic loop; and (v) they only have two, rather than six C-terminal membrane helices. CPx-type ATPases appear to have retained the energy conservation and phosphorylation mechanism, but their very different primary structure suggest major mechanistic differences. However, no three-dimensional structure is available for a CPx-type ATPase. In contrast, detailed structural information has been obtained for the calcium ATPase of the sarcoplasmic reticulum [15–19].

A unique property of eukaryotic copper ATPases is their relocalization in the cell under conditions of copper excess. Under low copper conditions, WNDP and related copper ATPases are localized in the *trans*-Golgi network where the enzymes pump copper into to lumen for the incorporation into cuproenzymes like ceruloplasmin or tyrosinase [20,21]. When cells are challenged with high copper levels, the copper ATPases traffic to a periplasmic vesicular compartment or to the plasma membrane. Copper-induced trafficking was first described for MNKP, but has also been described for WNDP and homologous enzymes [22]. The relocalization of the copper ATPases appears to serve in the secretion of excess copper, either into the bile by WNDP, or across the plasma membrane by MNKP. In patients with Wilson or Menkes disease, trafficking of the mutant enzymes is often impaired [23–25].

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Abbreviations: WNDP, Wilson disease protein; MNKP, Menkes ATPase; MBS, metal binding site; Δ1–5WNDP, six-histidine tagged Wilson ATPase only containing metal binding domain 6; SD, yeast minimal media

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The N-terminal MBSs are a typical feature of CPx-type ATPases. With the exception of a few bacterial enzymes which feature histidine-rich N-termini [26], the MBSs encompass a domain of 70–80 amino acids, containing a CxxC metal binding motif. The number of MBSs varies from one to two copies in bacterial enzymes to six copies in the human copper ATPases. MBSs are modular building blocks of the same βαββαβ structure [27–29] as the 8 kDa copper chaperones (e.g., HAH1, Atox1, Atx1, CopZ [30]). While it is clear that MBSs can bind copper(I) via their CxxC motif and interact with copper chaperones [31–33], their physiological function is still not entirely resolved. For WNDP, it was shown that only MBS6 closest to the membrane was required for function and copper-induced trafficking [34]. However, other studies showed that cooperative stimulation of the catalytic activity of the enzyme required the presence of both, MBS5 and MBS6 [35]. The situation appears to be similar for MNKP, where only MBS5 or 6 are required for function and trafficking [22]. In contrast to the human enzymes, neither of the two MBSs of the CopA copper ATPase of *Escherichia coli* appears to be required for function [36].

We here describe the establishment of a lean model system to investigate structure-function aspects of the human Wilson ATPase. The enzyme was truncated by MBS1–5, resulting in an enzyme only containing MBS6. A N-terminal histidine-tag was added for affinity purification of the enzyme. The resulting 103 kDa, six-histidine tagged Wilson ATPase only containing metal binding domain 6 (Δ1–5WNDP), was active in *Saccharomyces cerevisiae* and could be purified by Ni-NTA affinity chromatography. Induction by copper was necessary to solubilize the enzyme, suggesting copper induced trafficking to another membrane compartment. The purified ATPase could be reconstituted into proteoliposomes, was stimulated by copper, and had an approximate K_m of 0.2 mM for ATP and an ATPase activity of 7–8 nmol/min/mg. The system described here should provide a useful model for the in vitro study of WNDP.

2. Materials and methods

2.1. Materials

All chemicals were from Sigma-Aldrich or from Merck and were of analytical grade. Dodecylmaltoside was from Anawa Trading, Mono-Q column material from Amersham Pharmacia Biotech, and Ni-NTA Superflow from Qiagen. The following oligonucleotides were synthesized by Microsynth (Balgach, Switzerland): *insert1*, 5′-GATCCATGCCTGAACAGCATCACCATCACCATCACGATTACGATATCCCAACGACCGA AAACCTGTATTTTCAGGGCC; *insert2*, 5′-GTCGAGGCCCTGAAAATACAGGTTTTCGGTCGTTGGGATATCGTAATCGTGATGGTGATGGTGATGCTGTTCAGGCATG. The *S. cerevisiae* strains used in this study were as follows: BJ2168 (Mat a, prc1-407, pep4-3, prb1-1122, ura3-52, trp1, leu2, gal2) [37,38]; YSC7 (Mat α, Δccc2::LEU2, his3-200, trp1-101, ura3-52, ade5) [39].

2.2. Plasmid construction

Plasmid pG3A [40] containing the full length *ATP7B* gene was cut with BamHI and XhoI to excise MBS1–5. An insert encoding a 6xhistidine tag was generated by annealing primers *insert1* and *insert2* by mixing them in equimolar concentrations, heating them to 85 °C, and cooling them to room temperature over 2 h. This primer dimer, containing overhanging ends compatible to the *Bam*HI and *Xho*I ends of cut pG3A, was ligated with this vector to generate pG31. Plasmid pG31 contained a six-histidine tagged Wilson ATPase only containing metal binding domain 6 (Δ1–5WNDP). The plasmid was propagated in *E. coli* DH5α and was verified by commercial sequencing. The native plasmid used for these constructs, pG3 [41], was used as a control. All molecular biology procedures were conducted by published procedures [42].

Yeast transformation. The multiple protease resistant *S. cerevisiae* strain BJ2168 and the Δccc2 strain YSC7 were transformed with pG3 or pG31 by LiCl-mediated transformation as described [43]. Clones were selected on plates with minimal media containing uracil and leucine and were verified by PCR amplification. Standard methods were used for growth and phenotypic selection of yeast strains [44].

2.3. Expression and purification of Δ1–5WNDP

A starter culture of BJ2168 containing pG31 was grown aerobically in yeast minimal media (SD) containing uracil and leucine at 30 °C for 14 h. Of this starter culture, 2 ml were transferred to a fermenter containing 14 l of YPD media. The cells were grown for 14 h at 30 °C with 15 l/min of air and stirring at 400 rpm. The culture was induced with 5 mM $CuSO_4$ for 1 h and the cells harvested by centrifugation at $3000 \times g$ for 10 min. The following steps were performed with ice cold buffers. Cells were washed twice with buffer FP (50 mM Tris–SO_4, pH 7.4, 10% (v/v) glycerol, 200 mM K_2SO_4, 5 mM dithiothreitol, 0.024 g/l aprotinin, 0.065 g/l pepstatin A, 0.002 mM leupeptin, and 8.5 mM 4-aminobenzamidine) and resuspended in FP buffer (1 ml/g of wet cells). Cells were broken by three passages through a French press at 40 MPa. Cell debris was collected by centrifugation at $5000 \times g$ for 10 min and the supernatant was centrifuged at $100\,000 \times g$ for 1 h to collect cell membranes. The membrane pellet was resuspended in 1 ml of FP buffer per g of wet cells. The protein concentration was measured by the method of Bradford [45]. The membranes were extracted with dodecylmaltoside at a protein/detergent ratio of one. Insoluble material was collected by centrifugation at $100\,000 \times g$ for 1 h. The supernatant (membrane extract) was passed through a Ni-NTA Superflow column. Δ1–5WNDP was eluted with FP buffer containing 0.1% dodecylmaltoside and 200 mM imidazole. Final purification was achieved by gel filtration on a TSK3000G column in FP buffer containing 0.1% dodecylmaltoside.

2.4. Reconstitution of Δ1–5WNDP into liposome

Asolectin was purified as described [46], and dissolved in acetone at 50 mg/ml. For reconstitution, 10 mg of Asolectin in acetone were vacuum-dried and dissolved in 100 μl of 20% octylglucoside. The following steps were performed on ice. One millilitre of purified Δ1–5WNDP containing approximately 50 μg of protein was added to the Asolectin solution, followed immediately by dialyses against 200 volumes of 50 mM Tris–SO_4, pH 7.5, once for 2.5 h and once for 14 h. The vesicle density was checked by centrifuging vesicles without protein and vesicles containing Δ1–5WNDP on sucrose density gradients which were formed by freezing 25% (w/v) sucrose in 50 mM Tris–SO_4, pH 8.0, at 20 °C, followed by thawing at room temperature.

2.5. ATPase assay

Purified or reconstituted Δ1–5WNDP (5 μg) in assay mix (20 mM MES–Tris–SO_4, pH 6.0, 5 mM $MgSO_4$, 1 mM ascorbate, 0.1 mM tris(2-carboxyethyl)phosphine, and different amounts of $CuSO_4$ or BCA), was preincubated for 5 min at 37 °C. The ascorbate in the reaction reduced copper to copper(I). The reaction was started by the addition of 1.5 mM Na-ATP (or as required for K_m measurements), pH 6. Samples were removed at times 0, 10, 20 and 30 min and transferred to tubes containing 15 μl of 0.5 M Na-EDTA, pH 8. Released phosphate was determined in these samples by the colorimetric method of Lanzetta et al. [47].

3. Results

3.1. Expression of Δ1–5WNDP in yeast

For structural and functional work on WNDP, we constructed a plasmid (pG31) expressing a small, but functional variant of WNDP which contains a histidine tag for facile affinity purification. For this, we deleted 540 N-terminal amino acids containing MBS1–5 from WNDP and added a 6xhistidine tag by means of a synthetic, double-stranded oligonucleotide.

This resulted in a predicted product of 960 amino acids with a molecular weight of 103 kDa, called Δ1–5WNDP. To test if Δ1–5WNDP was functional in vivo, we transformed the yeast strain YSC7, which is defective in the CCC2 copper ATPase, with plasmid pG31, which expressed Δ1–5WNDP. In this stain, copper cannot be delivered to the *trans*-Golgi network where it would be required for the synthesis of Fet3. Fet3 is part of the high affinity iron uptake complex Fet3/Ftr1 at the plasma membrane. If no copper is incorporated into Fet3, the complex is not able to pump iron into the cell. Thus, a Δccc2 yeast strain cannot survive under copper- or iron-limiting conditions [39]. It had previously been shown that a defect in CCC2 can be complemented by heterologous copper ATPases such as human MNKP, WNDP, or the *Caenorhabditis elegans* copper ATPase [6,7,48].

Fig. 1 shows the growth response of the Δccc2 yeast strain YSC7 on media depleted of iron by different concentrations of ferrozine, and the effect of different vectors on the growth response. On normal media, all YSC7 strains grew to the same extent. With increasing amount of ferrozine, the Δccc2 yeast strain YSC7 as well as YSC7 transformed with the control vector pG3 did not survive due to iron limitation. Transformation of YSC7 with pG3A, the vector expressing full length WNDP, or transformation with pG31, the vector expressing Δ1–5WNDP, restored the ability of YSC7 to grow on iron-depleted media. Thus, WNDP as well as the truncated Δ1–5WNDP copper ATPase could complement the defect in copper homeostasis of the Δccc2 yeast strain. In fact, there was no noticeable difference in the complementation efficiency between WNDP and Δ1–5WNDP.

3.2. Purification of Δ1–5WNDP

For the biochemical analysis of Δ1–5WNDP, the protein was expressed in the protease deficient yeast strain BJ2168 transformed with pG31. In initial experiments, we were not able to extract Δ1–5WNDP from isolated yeast membranes with dodecylmaltoside or other detergents, even though the protein was expressed as determined on Western blots. Even the combination of detergents with 10% non-detergent sulfobetaines, which are powerful solubilizers, resulted in only marginal solubilization Δ1–5WNDP. We thus reasoned that the protein is localized in a highly inaccessible compartment and would traffic to a more soluble membrane fraction in the presence of copper. Indeed, when the cells were induced with 5 mM $CuSO_4$ for 1 h prior to harvesting, Δ1–5WNDP could easily be solubilized from the resultant membrane fraction with dodecylmaltoside. Induction of the cells with copper was therefore a key step in the solubilization of Δ1–5WNDP and suggests that the enzyme undergoes copper induced relocalization to a different membrane compartment. Affinity purification of Δ1–5WNDP over a Ni-NTA agarose column resulted in a protein of only 10–20% purity (Fig. 2). Further purification was achieved by anion exchange chromatography on a Mono-Q column, resulting in a Δ1–5WNDP preparation of approximately 75% purity. The protein band corresponding to an apparent molecular weight of 103 kDa was verified to be Δ1–5WNDP on Western blots with a polyclonal antibody specific for the WNDP C-terminal part [40]. The overall yield averaged 4 mg of purified protein from 14 l of yeast culture.

3.3. Reconstitution of Δ1–5WNDP

To be able to study the function of Δ1–5WNDP in membranes, we devised a reconstitution procedure for the generation of proteoliposomes. To this end, a detergent dialysis method was employed. Although dodecylmaltoside proved to be the detergent of choice for the purification of Δ1–5WNDP,

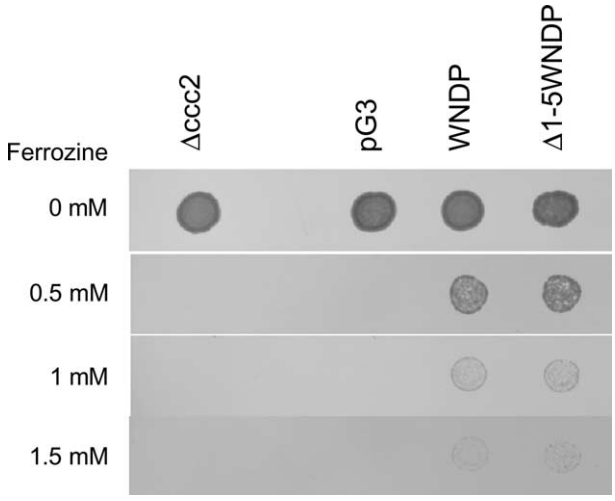

Fig. 1. Plate assay for the growth of different yeast strains in the presence of the iron chelator ferrozine. Yeast cells were plated on SD media containing the concentrations of ferrozine indicated in the Figure and grown aerobically for two days. Δccc2, untransformed control strain without copper ATPase; *pG3*, Δccc2 strain transformed with the control vector pG3; WNDP, Δccc2 strain transformed with pG3A expressing complete WNDP; Δ1–5WNDP, Δccc2 strain transformed with pG31 expressing Δ1–5WNDP.

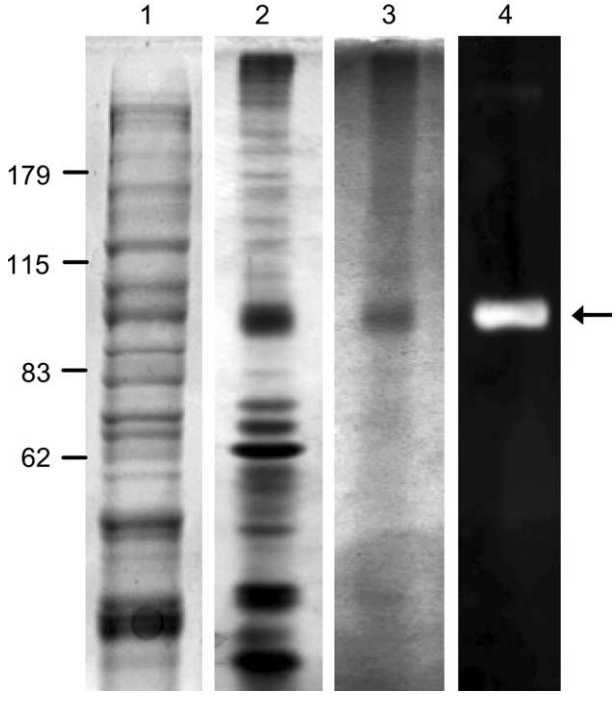

Fig. 2. Purification of Δ1–5WNDP. Detergent extract of membranes from cells expressing Δ1–5WNDP (lane 1) was purified on a Ni-NTA agarose column (lane 2), followed by purification on a Mono-Q anion exchange column (lane 3). The band of apparent molecular weight 103 kDa *(arrow)* was confirmed to by Δ1–5WNDP on a Western blot (lane 4). Other details were as outlined under Section 2.

it was not suitable for reconstitution. Because of its low critical micellar concentration of only 0.1–0.6 mM, it dialyses very slowly, which in turn impedes homogeneous vesicle formation. Octylglucoside with a critical micellar concentration of 20–25 mM is much better suited for the formation of proteoliposomes. It did, however, inactivate Δ1–5WNDP upon prolonged exposure and incubation times in octylglucoside had to be minimized. The efficiency of the reconstitution of Δ1–5WNDP into lipid vesicles was addressed by density gradient analysis of the proteoliposomes: protein-free vesicles are of lower density than those containing protein. Fig. 3 clearly shows that vesicles containing Δ1–5WNDP are denser than vesicles reconstituted without protein. The reconstitution of Δ1–5WNDP yielded only a small fraction of protein-free (lighter) liposomes (difficult to see in the reproduction of Fig. 3) and no heavy fractions, which would be indicative of protein aggregation. This suggests that the reconstitution of Δ1–5WNDP resulted in a relatively homogeneous proteoliposome population.

Purified, detergent solubilized Δ1–5WNDP displayed no significant ATPase activity. When reconstituted into proteoliposomes, a basal ATPase activity of approximately 4 nmol/min/mg was observed (Fig. 4). Depending on the preparation, this activity was stimulated to 7–8 nmol/min/mg by 10 μM added copper(I). At higher copper concentrations, the activity decreased to a level of around 5 nmol/min/mg at 100 μM copper. The basal ATPase activity was probably due to contaminating copper, but could only be reduced by 10–20% with 100 μM of the copper chelating agent bicinchoninic acid. Copper-stimulation of the ATPase activity suggests that the reconstituted enzyme was functionally competent and could be used for mechanistic studies. The affinity of Δ1–5WNDP for ATP was determined by measuring ATP hydrolysis at different concentrations of ATP in the presence of 2 mM copper and was approximately 0.2 mM (Fig. 5).

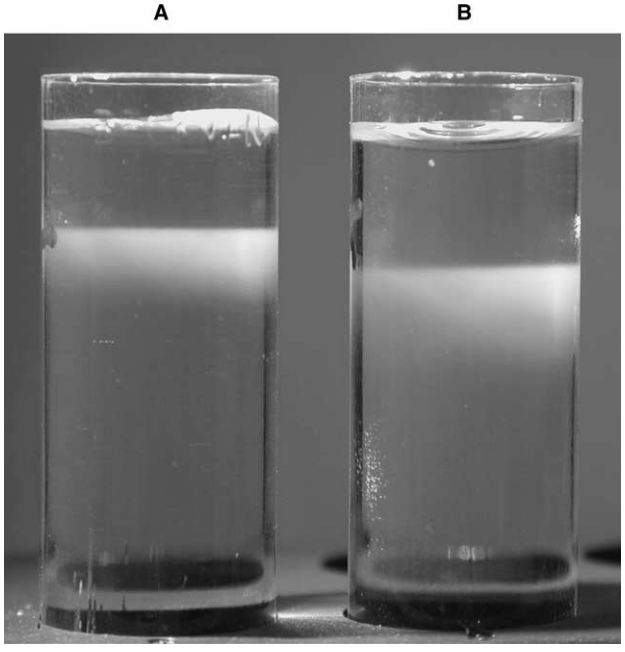

Fig. 3. Density of reconstituted vesicles. Vesicles reconstituted without protein (A) and with purified Δ1–5WNDP (B) were centrifuged on sucrose density gradients. The vesicle bands were visualized by illumination with white light from the side. Details of the procedure were as outline under Section 2.

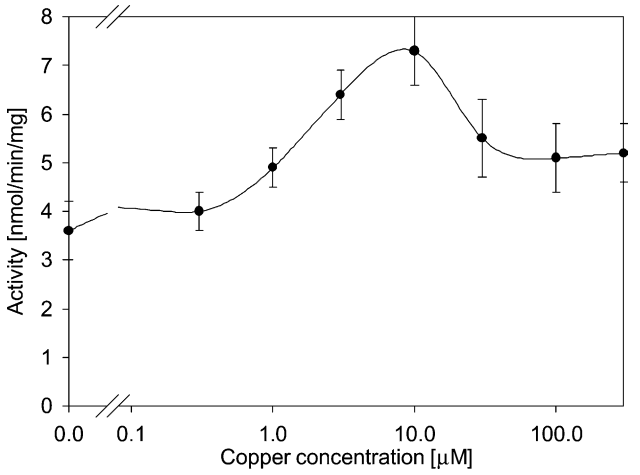

Fig. 4. Copper stimulation of Δ1–5WNDP in proteoliposomes. Vesicles containing Δ1–5WNDP were incubated with different concentrations of copper and the ATPase activity was measured as described under Section 2. The assays contained ascorbate, which reduces the added copper to copper(I).

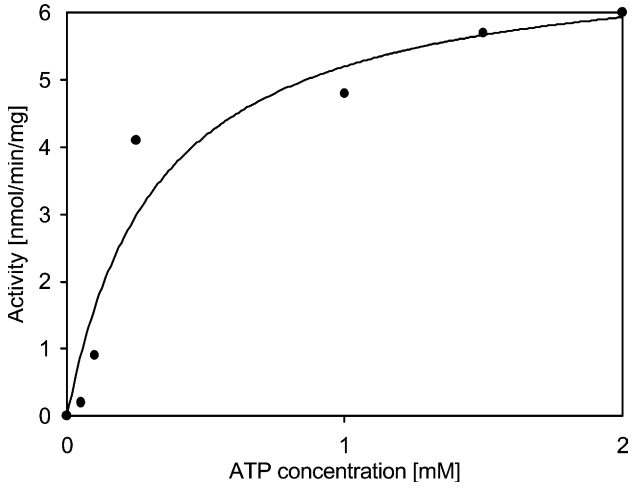

Fig. 5. Dependency of ATPase activity on the ATP concentration. The ATPase activity of Δ1–5WNDP in proteoliposomes was measured as a function of the ATP concentration. The activity was measured as described under Section 2.

In summary, this is the first demonstration of the purification and functional reconstitution of a human copper ATPase. Our studies also show that MNKP devoid of MBS1–5 is functionally competent not only in vivo, but also in a reconstituted in vitro system. The need for induction of the cells with copper to solubilize Δ1–5WNDP from the membrane fraction suggests copper-induced relocalization. Since protein expression in yeast is very economical, the system described here will be useful for structural and functional work on WNDP.

4. Discussion

We here describe the expression and in vitro analysis of the functional properties of WNDP, the human copper-transporting ATPase *ATP7B*. To make the protein easier to handle,

WNDP was truncated by its first five metal binding sites, resulting in Δ1–5WNDP. Using complementation of a Δccc2 yeast strain which is defective in the endogenous copper ATPase CCC2, we could show that Δ1–5WNDP was able to restore the ability of the Δccc2 yeast strain to grow on iron depleted media. Complementation by Δ1–5WNDP did not differ from complementation by full-length WNDP. This indicates that Δ1–5WNDP was fully functional. This observation is in accordance with the findings of Iida et al. [49], that MBSs1 to 5 are dispensable and only MBS6 is required for function of this copper ATPase in yeast.

For successful expression of Δ1–5WNDP, yeast strain BJ2168 which is deficient in multiple proteases had to be used. Δ1–5WNDP was present in low amounts in strain YSC7 and could not be purified from this host. Most likely, this has to be attributed to proteolytic degradation since the protease deficient host gave reasonable expression levels. Detergent extraction of Δ1–5WNDP from the *trans*-Golgi network, the presumed localization of the protein, turned out to be extremely difficult. Only aggressive detergents like sodium dodecyl sulfate could solubilize Δ1–5WNDP from the membrane fraction, resulting in an enzyme without activity. A key step in the purification of Δ1–5WNDP was the induction of the cells with copper before harvesting. This resulted in a membrane preparation from which Δ1–5WNDP could be solubilized with the mild detergent dodecylmaltoside, suggesting that Δ1–5WNDP translocated from the *trans*-Golgi network to another cellular compartment. Trafficking of the yeast CCC2 copper ATPase has not been described, but our observations suggest that there is copper-induced trafficking of Δ1–5WNDP in yeast and that this process does not require MBS1–5.

The reconstitution efficiency was tested by isopycnic centrifugation of the vesicles on sucrose density gradients. This test revealed that vesicles reconstituted with Δ1–5WNDP were of uniformly higher density than vesicles formed in the absence of protein. No Δ1–5WNDP could be detected at higher density, which indicates the absence of aggregated protein. Reconstituted Δ1–5WNDP had a basal activity of 4 nmol/min/mg. This could be due to contaminating copper, but also to a fraction of uncoupled ATPase. There was some reduction of the basal activity by 100 μM bicinchoninic acid, but a relatively high basal activity remained. Copper(I) stimulated the ATPase activity of Δ1–5WNDP to 7–8 nmol/min/mg with an EC_{50} for copper of 2 μM. The turnover appears relatively slow compared to non-heavy metal ATPases. Slow turnover may be a general property of eukaryotic copper ATPases. MNKP expressed in CHO cells was reported to pump copper at a rate of only 0.7 nmol/min/mg; however, this value was determined using native membrane vesicles [8]. Copper(I) concentrations above 10 μM inhibited the activity of Δ1–5WNDP, as had previously also been observed for CopB [50]. Tsivkovskii et al. using baculovirus-infected insect cells and Voskoboinik et al. [9,51] using transfected CHO cells reported K_m values for copper activation of WNDP of 2–5 μM, which is in good agreement with our findings.

The observed affinities for copper seem at odds with the findings of Rae et al. [52] which indicate that intracellular free copper is limited to less than one free copper ion per cell. This conclusion was based on the kinetics of cupration of yeast apo-superoxide dismutase. In a thermodynamic calibration of the interaction of the *E. coli* CueR regulator with copper in vitro, Changela et al. [53] found that it had zeptomolar (10^{-21} M) sensitivity to copper. This contrasts with findings using a biosensor for copper as well as an in vitro system to measure the induction of the *cop* operon of *Enterococcus hirae*. It was found that copper concentrations in the range of 2–5 μM initiated induction of the *cop* operon in vivo as well as in vitro and are thus ''relevant'' copper concentrations [54]. The issue of the 'copper concentration' in cells is far from resolved, but there are some know factors which can give rise to discrepancies. First, cellular chaperons can guide copper to ATPases, repressors, and cuproenzymes and thereby thermodynamically uncouple these systems from the free copper concentration. Secondly, copper ions form complexes with buffers, reductants and bio molecules, which makes the free copper concentration of in vitro systems difficult to control. Therefore, copper concentration/activity relationships can only be translated to *apparent* K_m values. This makes comparison of experiments from different labs virtually impossible. How much the *free* copper can actually depend on the concentration of proteins and copper-chelating agents in an assay has been dramatically demonstrated with a biosensor in *E. coli*, which presumably responds to *free* copper. Adding the same amount of copper to Luria broth or to a NaCl solution gave a 200-fold higher reading of the apparent free copper concentration in the salt solution [54].

The K_m for ATP of reconstituted Δ1–5WNDP was approximately 0.2 mM under the conditions used here. Using baculovirus- infected insect cells, Tsivkovskii et al. [51] reported a K_m for ATP of 1 μM for full-length WNDP and Voskoboinik et al. [9] using vesicles from transfected CHO cells, one of 10–15 μM. The high apparent K_m for ATP we measured for Δ1–5WNDP is probably not just a consequence of experimental differences. It could indicate functional disturbance of Δ1–5WNDP, either by the artificial lipid environment or by the truncation of MBS1–5. It has been shown that copper cooperatively stimulated the catalytic activity of WNDP and that this effect was lost in WNDP only possessing a functional MBS6, resulting in a 7–8-fold lower EC_{50} for the activation of catalytic phosphorylation by copper [35]. Cooperativity, which would be lost in Δ1–5WNDP, could also affect the affinity for ATP and could be the reason for the relatively high K_m we observed. In view of the prevailing cytoplasmic ATP concentrations of 1.4 mM in mammalian cells [55], the in vivo function of Δ1–5WNDP would, however, not be significantly impaired by its lower affinity for ATP.

WNDP has not been previously purified and reconstituted for functional studies. The system described here offers itself to structural and functional work as Δ1–5WNDP can be produced easily and at modest cost with this expression system.

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