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Molecular pathology of Wilson's disease: A brief

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Wilson disease (WD) is a rare autosomal recessive disorder of Cu metabolism linked to dysfunction of the Cu translocase ATP7B expressed in hepatocytes [1]. ATP7B is critical in the distribution and elimination of excess Cu from the organism. Malfunctioning of the translocase interferes with the distribution of Cu among organs and tissues and results in the imbalance between Cu absorption and excretion, thus causing Cu toxicosis.

The levels of Cu regulate the distribution and traffic of ATP7B in the hepatocyte. While at normal Cu concentrations, ATP7B remains in the TGN (trans-Golgi network) to supply Cu to the newly synthesized cuproproteins; at Cu concentrations exceeding the physiological limits it is transferred to the membranes of the bile canaliculi to dissipate the toxic levels of Cu [2].

Proper cellular distribution and functioning of ATP7B requires the coordinated interaction between its separate modules. Whereas the six Cu^+-binding domains in its long N-cytoplasmic domain play a major role in the acceptance of Cu^+ and in controlling the phosphorylation, traffic, and activity of the translocase, the clustering of its eight transmembrane helixes forms the Cu^+ pore, and the connecting loops in the cytoplasmic side are involved in the cyclic phosphorylation that controls the activity of the translocase (Fig. 1A and B) [3]. In addition, there is evidence that the C-cytoplasmic domain may also play an important role in the traffic and cellular distribution of the translocase [3,4].

Over 300 mutations in the ATP7B gene have been associated with WD, the majority missense mutations (60%). Mutations affecting to different extents the binding of Cu, the cyclic phosphorylation, traffic, and posttranslational modifications of ATP7B as well as its physiological interaction with other proteins, may potentially interfere with the dual role of ATP7B in excreting excess Cu into the bile and in the biosynthesis of the ceruloplasmin essential for proper iron (Fe) metabolism.

The most prevailing mutations in Wilson patients are H1069Q/G in Europe and North America, and R778L in southeast Asia [5]. Though H1069 residue plays a critical role in the ATP-binding to the N-domain of ATP7B, the demonstration that the H1069Q mutant is retained and quickly degraded in the ER suggests that this is the primary cause of the WD. The same traffic defect has been found in studies of the R778L mutant [6]. Besides, the association of WD with more than 40 different mutations in the N-site of ATP7B and the fact that only four of these mutations affect residues implicated in ATP-binding, point to the importance of cooperation between a large number of residues in the construction of a functional N-domain. The disruption of ATP7B-trafficking and the hampering of its phosphorylation provokes its dysfunction in the hepatocyte and explains the inhibition of Cu elimination into the bile. Interestingly, the liver from patients with G943S and M769V mutations, that inhibit the apical trafficking of ATP7B without interfering with its retention in the TGN, produces normal ceruloplasmin levels and as a result may not develop severe CNS intoxication.

Clinical symptoms in WD include cirrhosis and chronic hepatitis that end in liver failure, neurological defects that course with parkinsonian symptoms and seizures, and psychiatric features [7]. The Kayser–Fleischer ring, a deposition of Cu visible as a golden ring in the periphery of the cornea, low serum levels of ceruloplasmin, and high levels of Cu in the urine are helpful in the diagnosis of the disease. The age of presentation of the WD syndrome, the predominance of hepatic versus neurological symptoms and their severity are strikingly variable. Excessive Cu-derived oxidants produced by free Cu^{2+} - catalyzed Fenton reactions and reduced superoxide dismutase and glutathione activities, appear to contribute decisively to the development and progression of liver abnormalities in WD [8]. Furthermore, the toxic accumulation of Cu and the development of hepatic abnormalities without neurological symptoms in a group of WD patients is mimicked in the Cu toxicosis developed by the Long-Evans cinnamon rat (5′ deletion) and the 'toxic milk' mouse. Genotype variations in the mutations may partly explain the variability behind the clinical symptoms but is also likely that this also results from the activity of modifier genes and gene-environment interactions. Moreover, increased binding of Wilson's disease ATP7B with COMMD1, a negative regulator of protein stability involved in the quality control of ATP7B and absent in Bedlington terriers suffering from copper toxicosis, results in a decrease of their stability that may result in the amplification of the mutation effects and partially explains the clinical heterogeneity observed in WD [9].

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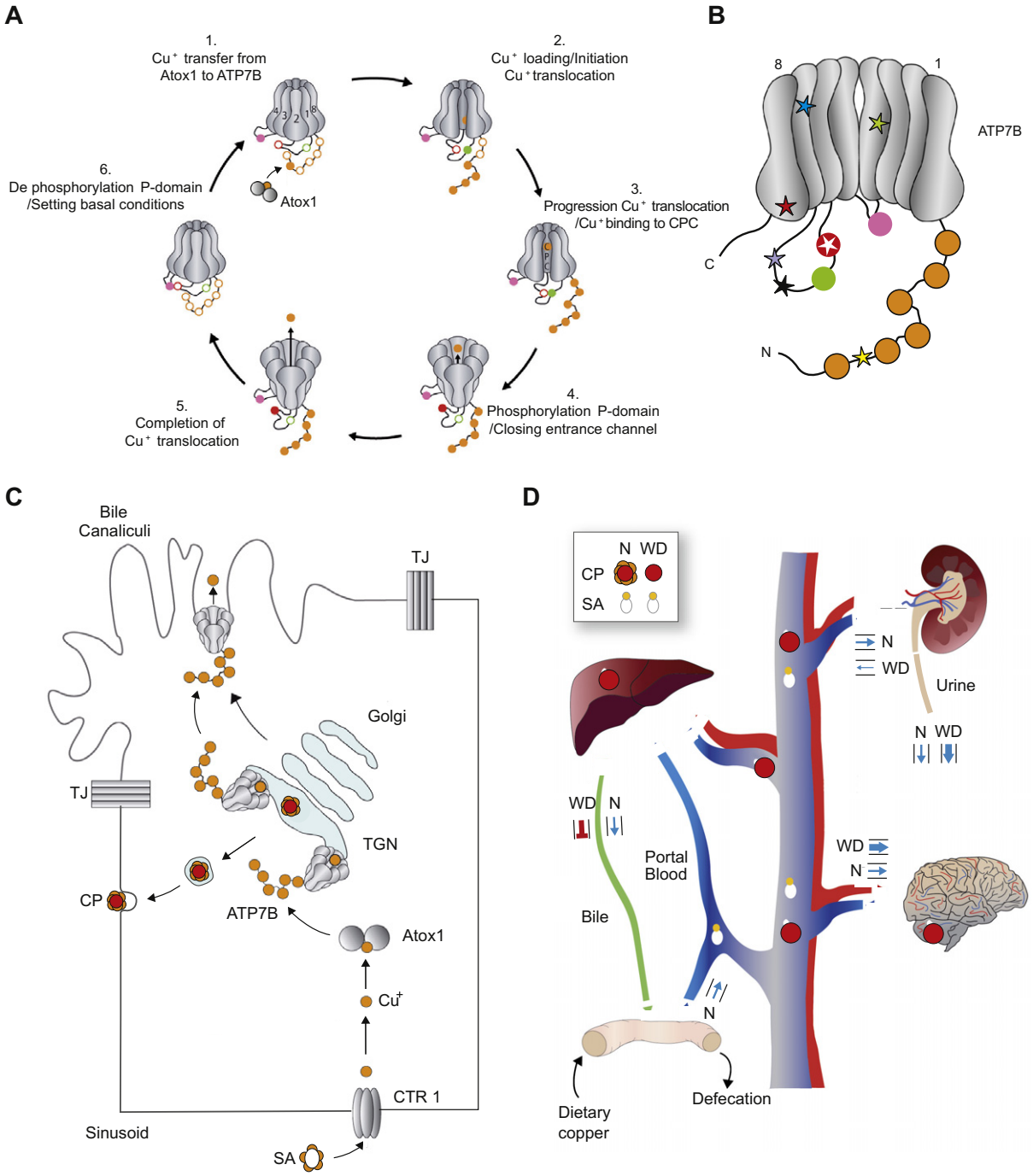

Fig. 1. Cyclic phosphorylation–dephosphorylation of ATP7B and vectorial transport of Cu through the ion channel. Five Cu binding domains (CBD) are aligned along the N-cytoplasmic end of ATP7B. The chaperone Atox1 transfers Cu^+ to the CBD2 [1]. The subsequent filling of CBDs1–4 with Cu^+ provokes structural changes that facilitate the binding of ATP to the N-site [2], bringing bound ATP in close proximity to the phosphorylation site [3] and facilitating the binding of a Cu^+ atom by the two juxtaposed Cys in the plane of the transmembrane domains TM1 and TM2 [3]. Next, filling of CBDs5 and 6 with Cu^+ favours the transfer of a Cu^+ atom to the CPC motif in TM6 [3]. Phosphorylation of the Asp residue in the P-site [4] closes the access to the ion channel from the cytoplasm and facilitates the reception of the translocated Cu^+ by the exofacial vestibule. Cu^+ is then released at the opposite side of the membrane and dephosphorylation of Asp-P by the phosphatase in the A-site [5] completes the phospho-dephosphorylation cycle [6] and resets the channel to basal conditions. (B) Point mutations (✦G85V, ✦R778L, ✦H1069Q, ✦C1104F, ✦V1262F, ✦G1341V, ✦S1363F) changes the levels, cellular distribution, and activity of ATP7B and results in Wilson disease (WD). (C) At physiological concentrations of Cu in the hepatocyte, ATP7B transfers the Cu^+ donated by Atox1 to the newly synthesized cuproproteins (i.e. ceruloplasmin, CP) moving through the *trans*-Golgi network (TGN). The increase in Cu concentration beyond physiological levels in the hepatocyte provokes the bulk translocation of ATP7B from the TGN to the membrane of the bile canaliculi where it mediates the elimination of excess Cu into the bile. Restoration of the physiological levels of Cu results in recycling of ATP7B to the TGN; TJ: Tight junction, SA: serum albumin. (D) Elimination of Cu by the hepatocyte into the bile is decreased in WD patients as compared to healthy individuals (N), causing Cu toxicosis. The main targets of toxic Cu are the brain and liver. Low resorption of Cu in the kidney results in high levels of Cu in the urine. The volume of Cu flow into organs and body fluids is indicated by the arrow size.

The capacity of Zn to induce metallothionein (MT) and the formation of tight MT–Cu complexes in the intestinal mucosa explains the effectiveness of the oral administration of Zn acetate in reducing dietary Cu absorption and the levels of intestinal haefestin, responses that ameliorate the clinical manifestations associated with Cu and iron accumulation in WD patients [10].

Conflict of Interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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