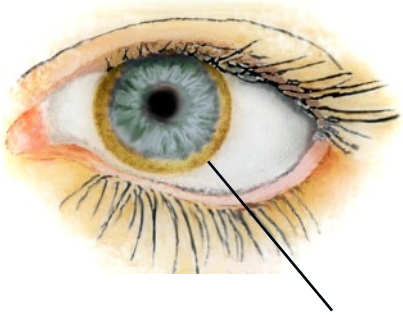
Wilson's disease and the copper ATPase transporters

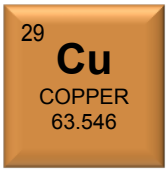
By Hsin-Yu Chang and Alex Mitchell

In the American TV series House, episode 'The Socratic Method', a mother seeming to suffer from schizophrenia is admitted to hospital. Her symptoms include hallucinations and liver malfunction. As usual, her condition is a puzzle for House's team, until the eventual 'Eureka moment'. The team solves the mystery through an eye examination. Kayser-Fleischer rings (copper-coloured circles in the cornea) are found in her eyes, fitting the diagnosis of Wilson's disease - a genetic disorder in which copper accumulates in tissue (mainly in the liver and brain).



Kayser-Fleischer ring

For most life forms, copper is an essential element. It is utilised by a variety of enzymes that play important roles in energy generation, oxygen transport and signal transduction. Since excess copper is toxic, several copper binding/transporting proteins that help maintain the copper balance within cells have evolved, and are preserved throughout evolution [1].



The Wilson's disease protein (ATP7B) is a good example of a conserved copper transporting protein. It is an ATP-driven copper pump that regulates copper homeostasis. Defects in human ATP7B cause an accumulation of copper, since the liver is unable to excrete the metal into the bile (Figure 1).

'Since excess copper is toxic, several copper binding/transporting proteins have evolved and are preserved throughout evolution.'

The InterPro database houses a wide variety of information on protein families and domains. Examining the database, information on the Wilson's disease protein and its homologues can be found in InterPro entries IPR001757 and its child entry IPR027256 (Table 1). From the description of IPR001757 , we know that ATP7B belongs to a broad family of membrane proteins that translocate ions across cellular membranes, using the energy created by ATP hydrolysis [2]. Transmembrane ATPases are classified into different types according to their functions, structures and the type of ions they transport. ATP7B is a Cu^+ transporting ATPase, which belongs to the IB subfamily of P-type ATPases (IPR027256) that is made up of ATPases involved in transporting Cu^+, Ag^+, Zn^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+} and Pb^{2+} ions. Family members are found in archaea, bacteria and eukaryotes.

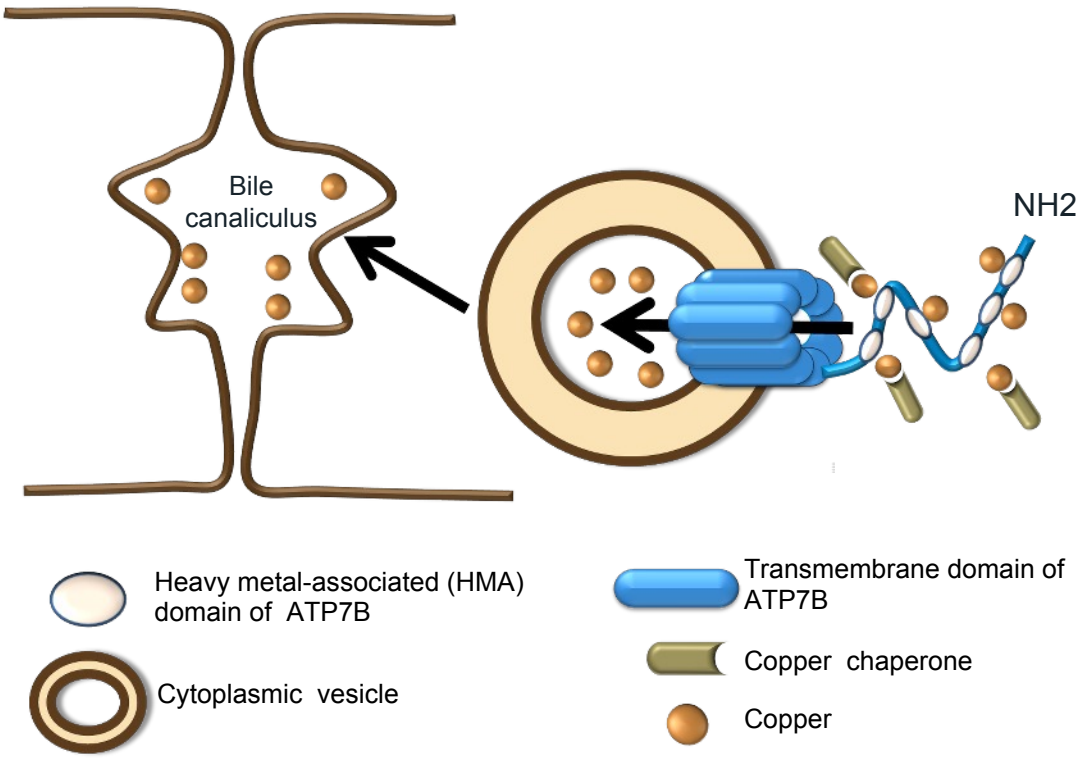


Figure 1. A model shows the Cu^+ transporting P-type ATPase, ATP7B, that excretes excess Cu^+ into the bile. Figure modified from Kim *et al*., Nature Chemical Biology 4, 176 - 185 (2008) [2].

Entry type	InterPro ID	Entry Name	Signatures	UniProt Protein Matches	PDB Protein Structure
Family F	IPR001757	Cation-transporting P-type ATPase	PR00119 PR00120 PTHR24093 TIGR01494	~43205	
Family F	IPR027256	Cation-transporting P-type ATPase, subfamily IB	PR00941 TIGR01525	~16898	
Domain D	IPR006121	Heavy metal-associated domain, HMA	PF00403 PS50846 SSF55008	~21387	1jww
Domain D	IPR008250	P-type ATPase, A domain	G3DSA:2.70.150.10 PF00122	~39433	2kij
Domain D	IPR023299	P-type ATPase, cytoplasmic domain N	G3DSA:3.40.1110.10 SSF81660	~37163	2arf
Site S	IPR018303	P-type ATPase, phosphorylation site	PS00154	~37529	

Table 1. Entries in InterPro that represent "cation-transporting P-type ATPase" and its related entries.

The structures of ATP7B and the closely-related family member ATP7A have been resolved [3,4,5,6]. They share a similar structure that consists of three major functional domains: the N-terminal heavy metal-associated domain (HMA), the A (actuate) domain and the N domain. The HMA domain (described by IPR006121) is the metal-binding domain, the A domain (described by IPR008250) has a regulatory function, while the N domain (described by IPR023299) is involved in nucleotide binding. In addition, a conserved phosphorylation site (described by IPR018303) is present, which can be found in all the P-type ATPases.

Using InterPro to compare ATP7B homologues across different species, we can see that the three major functional domains are conserved from bacteria to humans, but the number of HMA domains can vary. For instance, CopA from *E. coli* and Ccc2 from budding yeast only have two HMA domains, while ATP7B has six (Table 2). InterPro can also be used to predict the function of proteins that have not yet been experimentally characterised. For example, the database suggests that an uncharacterised protein (UniProt entry Q6H6Z1) from short grain rice is a member of the IB subfamily of the the P-type ATPase family, based on its matches to IPR001757 and IPR027256. Matches to other InterPro entries allow us to drill further into the

‘InterPro can be used to predict the functions of uncharacterised proteins’

potential function of this protein. We can see it has a domain organisation typical of other P-type ATPase IB family members, including a particular subclass of the HMA domain (also found in ATP7B) that is specifically involved

Species	Accession	Name	Domain organisation	Length (aa)
E. coli	Q59385	CopA		834
S. cerevisiae	P38995	Ccc2		1004
Homo sapiens	Q04656	ATP7A		1500
Homo sapiens	P35670	ATP7B		1465
A. thaliana	Q9S7J8	HMA7/RAN1		1001
A. thaliana	Q9SH30	HMA5		995
Oryza sativa subsp. Japonica	Q6H6Z1	Uncharacterised		1002

 Heavy metal associated (HMA) domain

 A (actuate) domain

 N domain, ATP binding (HAD like domain)

Table 2. Information about ATP7B and some of its homologues.

binding copper ions (see IPR006122).
It therefore seems likely that protein Q6H6Z1 is a copper transporting ATPase, similar to the Wilson's disease protein. Additional potential copper transporting ATPases from rice, such as A3AWA4, can also be predicted, based on similar matches to the InterPro database.

Accumulation of copper in rice is often toxic and can affect crop production [7]. Therefore it is important to identify and study copper transporting proteins in this species of plant. It will be interesting to know if InterPro's predictions are accurate and whether the proteins identified have a similar function to their homologues in Arabidopsis, such as HMA7/RAN1, which delivers copper to create functional hormone receptors involved in ethylene signalling [8].

'Accumulation of copper in rice is often toxic and can affect crop production'

It is fascinating that the Wilson's disease protein (ATP7B) and its homologues from bacteria to humans all play important roles in maintaining copper ion homeostasis. The study of these proteins has revealed the domains that underlie their function and provided useful information on the mechanisms of action of copper pumps.

Like the clinical tests Dr Gregory House calls upon to diagnose a patient's illness, InterPro, together with its member databases, can serve as a useful tool to help researchers spot potential new ATP7B homologues and identify their functions, hopefully guiding them to 'Eureka moments' of their own.

References

1. Gupta A and Lutsenko S. Evolution of copper transporting ATPases in eukaryotic organisms. Curr Genomics. 13 (2): 124-33. 2012 [PMID: 23024604].
2. Kim BE, Nevitt T, Thiele DJ. Mechanisms for copper acquisition, distribution and regulation. Nat Chem Biol. 4(3):176-85. 2008 [PMID: 18277979].
3. Argüello JM, Eren E, González-Guerrero M. The structure and function of heavy metal transport P1B-ATPases. Biometals. 20(3-4):233-48. 2007 [PMID: 17219055].
4. Banci L, Bertini I, Cantini F, Rosenzweig AC, Yatsunyk LA. Metal binding domains 3 and 4 of the Wilson disease protein: solution structure and interaction with the copper(I) chaperone HAH1. Biochemistry. 47(28):7423-9. 2008 [PMID: 18558714].
5. Banci L, Bertini I, Cantini F, Migliardi M, Natile G, Nushi F, Rosato A. Solution structures of the actuator domain of ATP7A and ATP7B, the Menkes and Wilson disease proteins. Biochemistry. 48(33):7849-55. 2009 [PMID: 19645496].
6. Dmitriev O, Tsivkovskii R, Abildgaard F, Morgan CT, Markley JL, Lutsenko S. Solution structure of the N-domain of Wilson disease protein: distinct nucleotide-binding environment and effects of disease mutations. Proc Natl Acad Sci U S A. 103(14):5302-7. 2006 [PMID: 16567646].

7. Yan YP, He JY, Zhu C, Cheng C, Pan XB, Sun ZY. Accumulation of copper in brown rice and effect of copper on rice growth and grain yield in different rice cultivars. Chemosphere. 65(10):1690-6. 2006 [PMID: 16844189].

8. Hirayama T, Kieber JJ, Hirayama N, Kogan M, Guzman P, Nourizadeh S, Alonso JM, Dailey WP, Dancis A, Ecker JR. RESPONSIVE-TO-ANTAGONIST1, a Menkes/Wilson disease-related copper transporter, is required for ethylene signaling in Arabidopsis. Cell. 97(3):383-93. 1999 [PMID:10319818].

Useful Links

InterPro
http://www.ebi.ac.uk/interpro/

UniProt
http://www.uniprot.org

Let us know

If you have any comments on this article please contact:
interhelp@ebi.ac.uk