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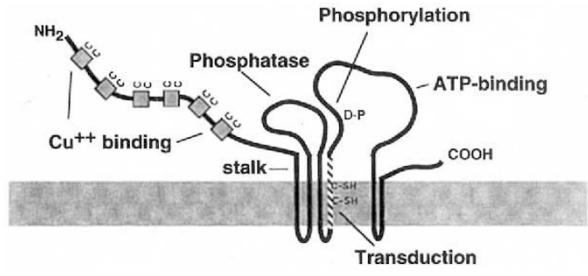
Cloning the Menkes disease gene

Three groups have successfully isolated the gene responsible for the X-linked Menkes disease, heralding great promise for our understanding of copper metabolism and for diagnosis of the disorder.

In 1962, John Menkes, a trainee neurologist at Columbia University in New York (who eight years earlier had described the first case of so-called Maple syrup urine disease) witnessed an infant with an unusual deteriorating brain disease and white, twisted, brittle hair[1]. The disease was evidently familial, and those affected lived for only a year or so. Initial suspicions as to the site of the defect focused on amino acid metabolism, but blood and serum measurements provided no clues. Ironically, ceruloplasmin levels monitored in a 1-week-old affected child also proved normal — unknown to Menkes at the time, they would subsequently decline, providing a telling clue to the underlying defect. But it was not until ten years later that David Danks noted that the unusual hair in Menkes disease, as it was then known, resembled the wool of sheep in Australia that suffered from copper deficiency[2]. (Copper is required for the cross-linking of amino acids in keratin and other connective tissue proteins.)

More recent biochemical studies have indicated that Menkes disease results from a copper-transport abnormality. Copper (Cu) ions are vital cofactors for at least half-a-dozen enzymes throughout the body, including lysyl oxidase, cytochrome *c* oxidase, superoxide dismutase and dopamine β-hydroxylase, and the majority of Menkes disease symptoms can be explained rather satisfactorily by deficiencies in these enzymes. Menkes patients, however, possess elevated amounts of Cu in most tissues (except the liver) suggesting that the intracellular transport of Cu is at fault. In particular, studies with cultured fibroblasts from Menkes patients have shown the efflux of Cu to be defective[3]. Speculation can now be firmly put to rest with the accounts in this month's *Nature Genetics* of the cloning of the defective Menkes disease gene on the X chromosome. Like most successes using the positional cloning strategy[4], the characterization of Menkes patients with translocations (of the long arm of the X chromosome) provided the key to finding the gene. And, fortunately, the complete structure of the Menkes gene product (see figure) contains a bounty of information for those seeking to understand ion transport and the role of heavy metals in cellular function.



Predicted structure of the Menkes disease protein — a P-type ATPase (see ref. 5).

The gene, which has been independently isolated by the groups of Jane Gitschier and Seymour Packman (University of California, San Francisco), Anthony Monaco (Institute of Molecular Medicine, Oxford) and Thomas Glover (University of Michigan), detects an 8.5 kilobase (kb) messenger RNA in most tissues examined[5–7] (although notably only minute levels are found in the liver). But in 23 out of 32 Menkes patients examined in all, the RNA was either qualitatively or quantitatively abnormal[5–7]. The Oxford group also found that 16 of 100 patients had various non-overlapping deletions of the gene[6].

The full-length complementary DNA sequence obtained by the San Francisco team reveals a predicted 1,500-amino acid protein containing six transmembrane domains[5]. Database homologies indicate that the Menkes gene product is a member of a cation-transporting P-type ATPase subfamily. The greatest resemblance is to the Cu export protein of *Enterococcus hirae*, but there are significant similarities to other prokaryotic cation transporters including those for cadmium and potassium. The predicted structure contains typical P-type ATPase ATP-binding, phosphorylation and phosphatase domains, as well as a cation channel. The phosphorylation domain contains a conserved aspartic acid residue which is thought to undergo a round of phosphorylation during the transport cycle. The protein also possesses a conserved proline residue (flanked by conserved cysteine residues) thought to be involved in energy transduction and ion binding. The N terminus contains six 23-residue repeats, each containing a Cys-X-X-Cys motif and probably involved in copper binding.

The cloning of the Menkes gene raises many intriguing questions. Could Wilson's disease, another hereditary disorder of Cu metabolism in which the liver accumulates Cu while other tissues are relatively unaffected, be attributable to a defect in a related liver-specific transporter? How similar is the gene responsible for *mottled*, the murine homologue of Menkes disease? And could the Menkes gene also be responsible for another disorder, X-linked cutis laxa (occipital horn syndrome), a connective tissue disorder featuring bladder abnormalities and bony 'horns' for which Cu abnormalities have been described? The San Francisco group have found that cultured fibroblasts from two cutis laxa patients are virtually lacking the Menkes gene transcript (although Southern analysis of the DNA appears normal)[8]. This suggests that the two disorders are indeed allelic, although interestingly, Menkes disease is also commonly associated with severe reductions in RNA levels. Finally, the cloned gene should greatly facilitate diagnosis of Menkes disease, which currently is performed biochemically in just two centres worldwide[7]. Although a severe disorder, early detection and copper histidine replacement therapy on a daily basis offer a promising form of treatment[9].

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Also in this month's *Nature Genetics*: expression studies of the fragile X syndrome gene, *FMR-1*, and a putative point mutation in a fragile X patient; mutations of the neurofibromatosis-1 gene in neuroblastoma; and the problems of defining genes in psychiatric disorders as evidenced by the case of X-linked manic depression.

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