Bad chaperone

Defects in microtubules are associated with a number of human diseases. Now, a chaperone protein that helps create microtubules is pinpointed in an inherited disorder.

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The Human Genome Project has greatly increased the pace of discovery of the causes of genetic disorders. In a recent example of this, eight research groups in six countries have combined to find the genetic lesion that causes a devastating autosomal recessive disease found mainly in people of Middle Eastern descent. Their findings are described in the November issue of *Nature Genetics*[1]. The causative mutation was found to be in the gene *TBCE*, encoding tubulin chaperone E, one of the chaperone proteins involved in the production of tubulin. In the same issue Martin *et al.*[2] report that a different mutation found in *Tbce* in mice results in another disease, progressive motor neuropathy.

The human disease is called HRD (hypoparathyroidism, mental retardation and facial dysmorphism) or Sanjad–Sakati syndrome. When patients also have osteosclerosis (bone thickening) and recurrent bacterial infections, they are classified as having Kenny–Caffrey syndrome. However, not only are these two syndromes caused by mutations in the same locus, they are caused by the same founder mutation[3]; all of the Middle Eastern patients are the descendents of one individual in which the mutation occurred. Sequencing of mutant and normal copies of the disease-causing gene show that the disease results from a 12-base-pair deletion, leading to the expression of TBCE protein lacking 4 amino acids in a putative tubulin-binding domain. A set of twins from Belgium with similar symptoms express a truncated form of the TBCE protein, further demonstrating that loss or alteration of the functioning of this protein is the cause of the wide-ranging developmental defects associated with these syndromes.

Tubulin production is a tightly regulated process involving many different chaperone proteins, some acting only on tubulin and some acting more generally (Fig. 1). Chaperone proteins were discovered about 15 years ago, by several groups, and knowledge of their number and roles keeps expanding[4,5]. They are defined as proteins that assist in the maturation of other proteins, especially in the prevention of illicit interactions between newly synthesized proteins that are not fully folded and are therefore sticky. At least seven different chaperones assist in the synthesis and quality control of tubulin[6] (Fig. 1). Prefoldin binds tubulin subunits as they are synthesized and hands them off to chaperonin, which helps them to fold to their native conformational state in an ATP-dependent reaction. Then the tubulin-specific chaperone proteins A, B, C and D take over, as well as the protein encoded by *TCBE*, chaperone E. These five chaperones work together to dimerize the tubulin subunits, releasing only those able to hydrolyze GTP, a reaction that is coupled to tubulin polymerization. Biochemical and genetic studies of these chaperones strongly suggest that they also play a role in degrading defective tubulin subunits and in regulating tubulin polymerization by causing tubulin to hydrolyze GTP, rendering it incapable of polymerization.

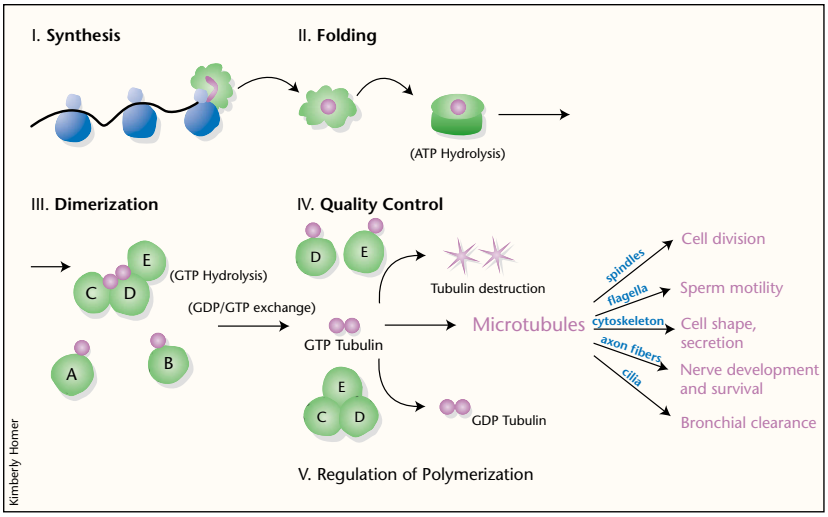

Fig. 1 Tubulin chaperones. The chaperone proteins prefoldin and chaperonin assist in the folding of newly synthesized tubulin subunits. The tubulin-specific chaperone proteins then act to dimerize α- and β-tubulin subunits, and, possibly regulate their polymerization into microtubules and degrade non-native or unneeded tubulins. Chaperone E is encoded by *TBCE*; mutations in *TBCE* give rise to HRD/Sanjad–Sakati syndrome in humans and progressive motor neuropathy in mice.

Tubulin is needed for many essential life processes, including cell division and proper organelle positioning in the cell. The two new studies present the first data on the genetics of tubulin-specific chaperones in animals. Because chaperones are required to produce tubulin, most mutations in the genes encoding these proteins are probably lethal at a very early stage of embryogenesis. This is true in plants, where mutations of the genes encoding tubulin-specific chaperones B, C, D and E result in large misshapen embryos unable to divide[7]. Therefore, the genetic lesions in *TBCE* which cause HRD/Sanjad–Sakati syndrome in humans and progressive motor neuropathy in mouse could not totally abrogate the function of tubulin-specific chaperone E. Both of these mutations probably leave the tubulin-specific chaperone E with differently impaired functions.

In the human disease, the researchers show that the 12-base-pair deletion mutation results in decreased microtubule density and the disorganization of the cytoskeleton (Fig. 2) and secretory apparatus. This has severe consequences for the development of these individuals. What is unexpected though, is that a tubulin deficit can give rise specifically to hypoparathyroidism in humans, and that a different mutation in the same

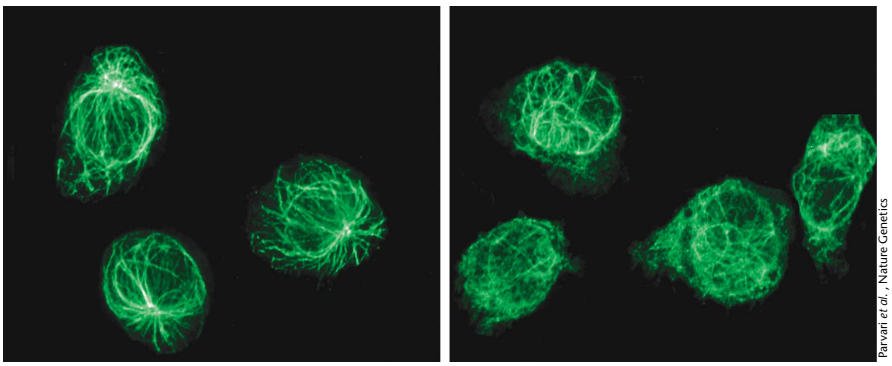

Fig. 2 Individuals with Kenney-Caffey syndrome have cells with abnormal tubulin architecture. ***a***, Organized tubulin in lymphoblastoid cells of a healthy individual. ***b***, Disorganized tubulin in cells from an affected individual.

gene results in a very different genetic disease in mice homozygous for the mutation[2].

The mouse mutation results in an alteration in the last encoded amino acid of murine chaperone E, which the authors show destabilizes the protein. The mice suffer from a progressive degeneration of their motor neurons, resulting in their death 4–6 weeks after birth. These mutant mice were thought to be a model for the human genetic disease spinal muscular atrophy, but the authors have shown that the latter disease is in fact caused by mutations in a different gene. When a construct expressing wild-type chaperone E was inserted into the mouse germline, the phenotype was reversed in homozygous mutant offspring carrying this construct. Although they have very different manifestations, the mouse and human diseases have several features in common. Both result in loss and disorganization of microtubules, and both result in growth retardation, small brains and defective spermatogenesis.

Tubulin protein sequences are highly conserved in all multicellular organisms, suggesting that almost any mutation in these essential building blocks is not tolerated. However, mutations in microtubule-associated proteins are the causes of several inherited human diseases, including frontal-lobe dementia with parkinsonism and lissencephaly (tau mutations)[8], Charcot–Marie–Tooth (*KIF1B* mutation) and Opitz syndrome (*MIDI* mutation). All of these diseases cause major brain impairment, consistent with the important role of microtubules in neuronal architecture and axonal transport. Several diseases, in addition to the tubulin-specific chaperone diseases discussed here, are caused by mutations in chaperones or putative chaperones, for example McKusick–Kaufman syndrome (mutations in the putative chaperonin, MKKS), spastic ataxia of Charlevoix–Saguenay (mutations in SACS, which is similar to hsp90) and desmin-related myopathy (mutations in human β-crystallin, a small heat-shock protein)[9]. In addition, as chaperones deal with misfolded proteins, they are involved in many diseases in which abnormal proteins are deposited as aggregates such as Huntington and Alzheimer disease[9]. Thus chaperones are considered potential therapeutic targets because of their ability to disaggregate and dispose of misfolded proteins.

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Circadian rhythm beats back cancer

The *Per2* gene helps measure the pace of the circadian rhythm—and now it appears that it also helps keep cancer at bay, according to a study in the October 4 Cell. Fu *et al.* suspected a connection to cancer when they found hyperplasia in the salivary glands of relatively young *Per2*-mutant mice. They next tested the animals' sensitivity to radiation, an indication of cancer susceptibility. Irradiation can damage the cells responsible for hair color. Indeed, after irradiation all of the *Per2* mutant mice developed prematurely gray hair (shown here) and a high frequency of lymphomas as compared to wild-type mice. Mutant mice also had aberrant temporal expression of genes involved in cell cycle regulation and tumor suppression. The researchers honed in on a mechanism for circadian control of one such gene, the p53 regulator c-*myc*. Its transcription is controlled directly by two PER2-controlled clock proteins. The results jibe with previous findings hinting at a link between circadian cycles and cancer; for example, women working the night shift appear to have an increased risk of breast cancer.

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