Cu^+-ATPases Brake System

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Copper (Cu^+) transport ATPases are characterized by cytoplasmic metal-binding repeats. Using cryo-electron microscopy (cryo-EM) of functionally intact Cu^+-ATPases and high-resolution structures of isolated domains, Wu et al. (2008) produced a model that explains how Cu^+ binding to cytoplasmic sites controls the enzyme transport rate.

Copper transport ATPases are members of the P-type ATPase superfamily of transmembrane proteins. These are responsible for creating ion gradients across the biological membranes at the expense of ATP hydrolysis (Axelsen and Palmgren, 1998). Their relevance is highlighted, for instance, by phenotypes observed for the Menkes' and Wilson's diseases, in which patients carry mutations in the corresponding genes. Cu^+-ATPases show significant functional and structural differences from other P-type ATPases: (1) The Cu^+ substrate accesses the transmembrane transport sites bound to specific chaperones (González-Guerrero and Argüello, 2008); (2) They present a reduced number of transmembrane segments in a singular arrangement; and (3) They have 1–6 cytoplasmic metal-binding domains in their N terminus (N-MBDs). Although most research on the molecular aspects of Cu^+-ATPases has focused on the role of N-MBDs, progress has been limited by the lack of a structural description of the intact Cu^+-ATPase molecule. The work of Wu et al. reported in this issue tackles this by providing an initial atomic representation of a functionally complete Cu^+-ATPase (Wu et al., 2008). The proposed structure, placing the N-MBDs in close contact with catalytic mobile domains, supports and integrates the available data into a plausible model for the self-inhibitory role of N-MBDs. Moreover, it provides a framework for postulating testable hypotheses on the molecular mechanism of Cu^+ transport.

The presence of repeated N-MBDs was detected in early work on P_{1B}-ATPase sequences, which showed them to be homologous to soluble Cu^+-chaperones (Lutsenko et al., 1997). Biochemical studies confirmed their metal-binding capability and showed that they exchange Cu^+ with specific soluble chaperones. These observations led to the hypothesis that N-MBDs might mediate ion transfer from the chaperone to the transmembrane metal-binding sites (Huffman and O'Halloran, 2000). However, recent evidence suggests that N-MBDs are unable to transfer Cu^+ to transport sites, but that it is the chaperones which upload the ion directly into membrane translocating sites (González-Guerrero and Argüello, 2008).

While cell biology studies of the mammalian Cu^+-ATPases have indicated that N-MBDs are responsible for Cu^+-dependent targeting of these proteins (Lutsenko et al., 2007), the role of N-MBDs on enzymatic (catalytic) function has remained somewhat controversial. For instance, the presence of N-MBDs in bacterial and archaeal proteins suggest a functional role independent of membrane targeting. Biochemical analysis of P_{1B}-ATPase mutants in which the metal-binding capability was removed from the MBDs indicated that the enzymes were functional, albeit with a reduced turnover rate (Argüello et al., 2007). On the other hand, truncation of the ATPase, fully removing the N-MBDs, yielded enzymes with higher enzyme turnover (González-Guerrero and Argüello, 2008). These results suggest a regulatory/self-inhibitory role for N-MBDs in controlling the turnover rate of the enzyme. Such a mechanism would require the interaction of N-MBDs with catalytic domains, as suggested by the early observations by Tsivkovskii et al. of Cu^+-dependent interaction between N-MBDs and the ATP-binding domain (Tsivkovskii et al., 2001).

Understanding how Cu^+ binding to N-MBDs controls the transport rate via domain interaction requires knowing the overall architecture and domain organization of these proteins. Full length P_{1B}-ATPases have been recalcitrant to crystallization. However, recently the high-resolution structures of cytoplasmic domains have been reported (Argüello et al., 2007; Lutsenko et al., 2007). Now, Stokes and coworkers, using a strategy that has proven successful for various membrane proteins, combined these atomic structures with a lower-resolution model produced by cryo-EM of archaeal Cu^+-dependent P_{IB} ATPase, CopA, tubular crystals to build pseudoatomic models of the protein. Interestingly, by comparing the structure of truncated proteins, the authors were able to localize the N-MBD, interacting with the actuator (A) and the ATP-binding domains. The model not only agrees with previous data, but provides an insight into the regulatory mechanism. Structures of the Ca^{2+}-ATPase have shown that a dramatic A-domain rotation couples the ion-binding and catalytic sites during the transport cycle and that pivoting of the nucleotide-binding (N)-domain accompanies ATP binding and phosphoryl transfer (Toyoshima and Inesi, 2004). Consequently, Stokes and his collaborators propose that in the absence of Cu^+ (condition in which CopA structures were obtained) the N-MBD would restrict A-domain and N-domain movement. Upon binding Cu^+, the N-MBDs would be displaced and thus, allow for the required motions of the catalytic domains. We think that it is quite likely that the N-MBDs mainly prevent the rotation of A-domain associated with gate opening and metal release. This rate-limiting step is affected (slowed down) when Cu^+-loading of N-MBDs is prevented by mutations of metal coordinating cysteine residues (Argüello et al., 2007). This can

be visualized as the N-MBDs acting as a mechanical brake of the transporting machinery by physically restricting the rate-limiting movement.

The described model certainly leaves several structural questions unanswered. How, or where, does the Cu^{+}-loaded chaperone interact with the ATPase? Can the arrangement of transmembrane segments be better defined? How are multiple N-MBDs accommodated in the structure? Interestingly, these issues are within the reach of cryo-EM approaches. As in the case of the role of N-MBDs, addressing these would have a significant impact in the field.

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Insights into the Kinetochore

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The Ndc80 complex is a core component of the kinetochore, which links chromosomes to microtubules. Recently, Ciferri et al. (2008) published an atomic-level structure of the complex with implications for kinetochore architecture and for the generation and control of chromosome movements during mitosis.

Background

All life depends on the accurate distribution of duplicated DNA during cell division. In eukaryotic cells, this process is carried out by an integrated molecular machine, the mitotic spindle, named in the 1800s for its similarity to a part of the spinning wheel from that time. Beyond its shape, however, the mitotic spindle bears little resemblance to its yarn-spinning namesake. It has four key components, each a fascinating molecular machine in its own right (Figure 1A): (1) the replicated chromosomes, or sister chromatids, which are held together in pairs until the spindle is fully assembled; (2) the spindle poles, which organize the microtubules; (3) the microtubule fibers, which extend from the spindle poles; and (4) the kinetochores, specialized structures on each chromosome where the microtubules attach.

Kinetochores form a bridge between the chromosomes and the microtubule fibers, and they are at the nexus of the mitotic process (for review, see Cheeseman and Desai, 2008). Kinetochores are able to convert the energy from microtubule depolymerization into chromosome movement. The mitotic checkpoint, a process which prevents premature chromatid separation, acts through the kinetochore (for a review, see Musacchio and Salmon, 2007). The checkpoint can detect even a single unattached kinetochore and delay chromatid separation until all are attached. In response to incorrectly attached kinetochores, the checkpoint also induces corrective detachment. As expected for a molecular machine with so many functions, the kinetochore is a focal point for regulation, which occurs through phosphorylation, sumoylation, and methylation of its components. Uncovering how the kinetochore works is central to understanding mitosis.

Spindle microtubules are constantly growing and shortening, and biologists have long wondered how kinetochores stay attached to these dynamic filaments. Time-lapse movies show that kinetochores and their associated chromosomes move continually back-and-forth as the microtubules polymerize and depolymerize under their grip. Several models explaining this dynamic attachment proposed in the 1980s (Hill, 1985; Koshland et al., 1988) are becoming directly testable. Through a combination of genetics and biochemistry, we now know that the kinetochore is a collection of at least 60 proteins arranged into subcomplexes (Cheeseman and Desai, 2008). An increasing number of these subcomplexes can be produced in recombinant form in large quantities, paving the way for biochemical and biophysical interrogation, for structural studies, and possibly for complete reconstitution of active kinetochores from pure components. While a few EM structures are available (Davis and Wordeman, 2007; Wang et al., 2007), atomic-level structural information has been challenging to acquire. Now, in an important advance,