BIOCHEMISTRY

Delivering Copper Inside Yeast and Human Cells

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Copper is absolutely required for aerobic life and yet, paradoxically, is highly toxic. Within the living cell, it coexists with high concentrations of electron-rich molecules such as thiols or ascorbate that are essential to life. But, in the laboratory, it is a superb catalyst for the oxidative destruction of those same molecules. This apparent contradiction has been rationalized by assuming that Cu, like other redox-active metals, is sequestered in nonreactive forms as it is transported into cells and moves through cellular compartments. However, the agents of such trafficking and the mechanisms of delivery of Cu to its final destinations have, until recently, remained largely unknown. Our knowledge of this area has increased substantially during the past 2 years with the identification of two proteins involved in Cu trafficking in yeast: Atx1 (*1*) and Cox17 (*2*). Two reports now add even more to our knowledge in this area. The first, by Pufahl *et al.* on page 853 of this issue (*3*), concerns the mechanism of in vivo Cu transfer by Atx1; and the second, by Culotta *et al.* (*4*), reports the identification of a similar Cu-trafficking function for Lys7 in catalyzing Cu incorporation into apo–copper-zinc superoxide dismutase (CuZnSOD) in yeast.

The uptake of Cu in yeast begins with reduction by one of several plasma membrane reductases. The reduced Cu is then transported across the membrane by the high-affinity Cu transporter Ctr1 (*5*) (see the figure). Three different proteins have been identified that transport Cu from Ctr1 to three different cellular locations: Cox17 (*2*) guides Cu to the mitochondria for insertion into cytochrome c oxidase (CCO), the terminal oxidase of the respiratory chain; Lys7 (*4*) targets Cu to CuZnSOD, a primary antioxidant enzyme in the cytosol; and Atx1 (*1*) directs Cu to a post-Golgi compartment, by way of Ccc2 (*6*), a P-type adenosine triphosphatase (ATPase) transmembrane Cu transporter, for final insertion into Fet3, a multicopper oxidase essential for high-affinity iron uptake.

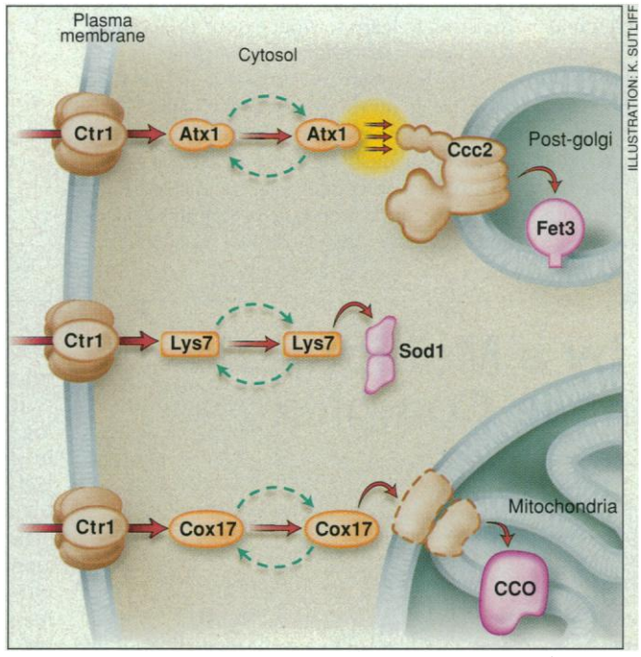

ILLUSTRATION: K. SUTLIFF

Copper trafficking in yeast. Cu in the form of Cu(I) is transported across the plasma membrane by Ctr1 and then transferred to small, soluble cytoplasmic Cu transporters, or Cu chaperones; the three currently known Cu transporters—Atx1, Lys7, and Cox17—are shown in orange. Each feeds Cu to a specific protein—Fet3, Sod1 (CuZnSOD), and CCO (cytochrome c oxidase)—in a different cellular compartment. A physical interaction between the components of these pathways (yellow highlighting) has been directly demonstrated only for Atx1 and Ccc2; the precise mechanism of the transfer to the other proteins is unknown and could involve unidentified intervening proteins. In mitochondria, more components are likely (dashed brown boxes); Cu must cross at least one membrane and a second aqueous compartment (the intermembrane space) to reach its final destination. One of these components may be Sco1, an inner membrane protein involved in CCO assembly. Sco1 overexpression can rescue yeast lacking Cox17 (*12*). The copper pathways are indicated by red arrows. Green dashed arrows, the diffusion of the Cu chaperones in the cytoplasm; membrane transporters, brown; final target proteins for Cu, pink.

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It is assumed that molecular recognition and binding events are essential parts of the individual Cu-trafficking pathways because of the apparent specificity each protein shows for its final site of Cu delivery. Nevertheless, inhibiting Cu delivery by genetically eliminating one of these proteins does not in itself prove that a particular protein interacts directly either with Ctr1 or with the next (genetically defined) acceptor of Cu delivery along that pathway. Pufahl *et al.* (*3*) now present the first direct evidence for an interaction and recognition event in Cu transfer from a Cu-trafficking protein to the acceptor protein with their finding, based on a yeast two-hybrid experiment, that Atx1 interacts directly with the metal-binding domain of Ccc2 in vivo (see the figure). In their physical studies of the Cu-binding site of Atx1, Pufahl *et al.* also provide the first characterization of the metal-binding motif, Met-X-Cys-X-X-Cys (X, any residue), that is found in these proteins involved in the transport and transfer of metal ions. They demonstrate that both a three-coordinate and a two-coordinate geometry are possible for such a site, suggesting the very reasonable possibility that interconversion between the two geometries plays a role in the mechanism of metal ion exchange from one protein to another. The Cu chemistry of Atx1 is likely to be similar to that of the Atx1-like domains in several other Cu proteins including the Menkes and Wilson disease ATPases.

This information about Cu metabolism in yeast gains even more significance because the three soluble cytoplasmic Cu chaperones as well as two membrane transporters have human homologs that are functional when they replace the natural yeast protein: yeast Atx1/human Hah1 (*7*), yeast Lys7/human Ccs1 (*4*), yeast Cox17/human Cox17 (*8*), yeast Ccc2/human Wilson disease protein (*9*), and yeast Ctr1/human Ctr1 (*10*). In addition, the multicopper oxidase Fet3 is homologous to human ceruloplasmin (*6*). These findings suggest strongly that many aspects of the mechanisms of Cu and iron homeostasis in yeast and humans will prove to be very similar.

The Cu transport mechanisms described in the figure are high-affinity pathways, active in conditions of low Cu concentration,

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and some of them can be entirely bypassed when there are high concentrations of Cu salts in the medium. Thus, yeast strains missing the gene for Cox17 cannot respire in normal growth media because CCO is Cu deficient, but are rescued when the medium is made 0.4% $CuSO_4$ (*2*). Likewise, increasing the Cu concentration in the medium allows Cu to be delivered to Fet3 in yeast strains missing the gene for Atx1 (*1*). These results indicate that neither Cox17 nor Atx1 is required for proper Cu trafficking when Cu levels are high and that their presence is not absolutely required to detoxify Cu. The observation that high levels of Cu in the growth medium can under some circumstances be beneficial to yeast is counterintuitive but reminiscent of the ability of similarly high levels of Cu to enable strains lacking CuZnSOD to grow well in air, as long as metallothionein is present (*11*). Metallothionein is a key player in Cu detoxification for yeast, probably acting as a Cu buffer to keep intracellular "free" Cu concentrations low; its synthesis is induced by Cu.

Multiple Cu-binding equilibria must be present in the cell, since Cu-binding proteins such as metallothionein, Atx1, Cox17, and Lys7 are all cytosolic and must presumably compete for available Cu. It will be interesting to learn to what extent this competition regulates cellular Cu distribution, and whether competitive success is determined by kinetic or thermodynamic factors. Although many questions remain, the convergence of chemistry, biochemistry, and genetics on this problem has set the stage for a complete understanding of cellular copper and iron metabolism, from the molecular details of the intermolecular transfer reactions to the genetic control of the relevant proteins in both yeast and humans.

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SIGNAL TRANSDUCTION

Catalysis by a Multiprotein IκB Kinase Complex

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The transcriptional activator protein NF-κB mediates key immune and inflammatory responses (*1*). NF-κB, present in the cytoplasm of most cell types, is normally bound to a member of a family of inhibitor proteins known as IκB (Inhibitor κB). The best-characterized member of this family, IκB-α, binds the p50/p65 heterodimer of NF-κB in the cytoplasm. When cells are exposed to inducers of NF-κB, such as the cytokines tumor necrosis factor–α (TNF-α) or interleukin-1 (IL-1), two serine residues of IκB-α (Ser^{32} and Ser^{36}) are specifically phosphorylated. This phosphorylation is a signal for ubiquitination and degradation of IκB-α by the 26S proteasome (*2*, *3*). NF-κB is thus released to translocate to the nucleus and activate transcription of target genes.

Because NF-κB can be activated by an extraordinarily large number of different signals, ranging from ultraviolet irradiation to T cell activation (*1*), the mechanism by which these signals converge on IκB-α is of wide interest. During the past 2 years, many components from certain signaling pathways that lead to activation of NF-κB (*3*, *4*) have been described. The recent identification of a high molecular mass IκB kinase complex (*5*) and the identification of two unusual IκB kinases, reported in this issue on pages 860 and 866 (*6*, *7*), and elsewhere (*8*, *9*) now provide a framework for resolving the problem of integrating multiple NF-κB signaling pathways.

A number of cell-surface proteins interact specifically with the intracellular domains of the TNF-α and IL-1 receptors and are intermediates in the activation of NF-κB (*3*, *4*). One of these, TRAF2, is recruited to the TNF-α receptor and another, TRAF6, is recruited to the IL-1 receptor (*10*); both TRAFs interact with a MAP kinase kinase kinase (MAPKKK) known as NIK (*11*, *12*). Cell transfection experiments with wild-type and dominant-negative mutants of NIK suggest that this kinase is required for the TNF-α– and IL-1–dependent activation of NF-κB (*11*, *12*). Another MAPKKK, MEKK1, has been implicated in the TNF-α pathway, but its role is more controversial and its mechanism of activation not well understood (*12–15*).

Many kinases have been suggested to phosphorylate IκB-α, but until recently, none was shown to phosphorylate the critical serine residues. Last year, a kinase activity capable of specifically phosphorylating Ser^{32} and Ser^{36} of IκB-α was identified in cytoplasmic extracts from uninduced HeLa cells (*5*). Remarkably, this kinase fractionated as high molecular mass complex (~700 kD). Although it is inactive alone, this complex can be activated in vitro by treatment with purified recombinant MEKK1, directly implicating a MAPKKK in its activation (*5*, *14*). Quite unexpectedly, this complex could also be activated independently by ubiquitination (*5*, *14*). Finally, an already active IκB kinase complex of approximately the same size could be detected in extracts from TNF-α–induced HeLa cells (*14*). These studies, however, did not identify a specific protein kinase that directly interacts with and phosphorylates IκB-α.

A major breakthrough in the search for IκB kinases is now provided by three different groups, which report the identification and cloning of two closely related protein kinases that appear to directly phosphorylate IκB-α (*6–9*). Both Karin's group at University of California in San Diego and Mercurio, Rao, and their co-workers at Signal Pharmaceuticals and the Harvard Medical School approached the problem by purifying the high molecular mass IκB kinase complex from TNF-α–induced cells (size estimates from the different labs range from 500 to 900 kD) (*6–9*). Independently, Rothe, Goeddel, and their co-workers at Tularik identified one of the kinases by using the NIK kinase as bait in a yeast two-hybrid screen (*8*), and the other by virtue of

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