glycoprotein glomalin produced by arbuscular mycorrhizal fungi (another major group) is thought to reside for decades in the soil (*11*). It is not clear whether analogous compounds are constructed by other mycorrhizal fungi and under what conditions. Another open question is whether ericoid and ectomycorrhizal fungi conduct decomposition themselves. Many members of these groups have the physiological capacity to break down and take up soil organic material (*8*), which could ultimately result in the production of CO_2. Finally, the extent to which mycorrhizal fungi improve plant growth can also determine how much carbon is deposited in the soil via dead plant material. It is the sum of these three processes—deposition of mycorrhizal residues, decomposition by mycorrhizal fungi, and augmentation of plant growth—that determines how mycorrhizal fungi affect carbon storage.

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BIOCHEMISTRY

A Protease for the Ages

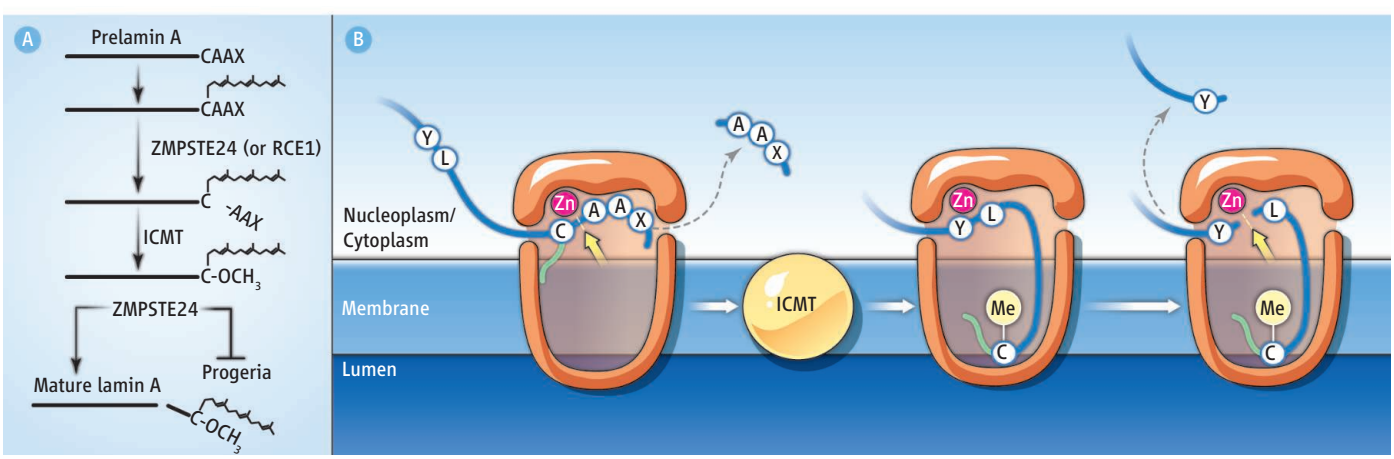
Structures of membrane metalloproteases provide the basis for understanding mutations associated with premature aging.

Susan Michaelis[1] and Christine A. Hrycyna[2]

Mutations in the genes encoding the nuclear scaffold protein lamin A or the zinc metalloprotease ZMPSTE24 cause the devastating premature aging disorder Hutchinson-Guilford progeria syndrome (HGPS) and the related progeroid disorders restrictive dermopathy (RD) and mandibuloacral dysplasia (MAD-B) (*1*–*4*). Children with HGPS, for example, manifest accelerated aging symptoms, including failure to thrive, hair loss, joint ailments, lipodystrophy, and cardiovascular disease, typically dying from the latter in their mid-teens. In all of these progeroid disorders, a persistently farnesylated and methylated form of lamin A is the "molecular culprit," exerting dominant-negative effects that promote aging-related symptoms (*1*). On pages 1604 and 1600 of this issue, Quigley *et al*. (*5*) and Pryor *et al*. (*6*) report the three-dimensional crystal structures of the human zinc metalloprotease ZMPSTE24 and its yeast homolog, Ste24p. These proteases play critical roles in two steps of the posttranslational maturation of human lamin A and the yeast mating pheromone **a**-factor, respectively (*7*–*9*). ZMPSTE24 and Ste24p are multispanning membrane proteins and as such, determining their structures by x-ray crystallography represents a substantial accomplishment. The structures should lead to a better understanding of how these enzymes function and how they are associated with aging.

The ZMPSTE24 substrate lamin A maintains the structural integrity of the nucleus. It is synthesized as a precursor, prelamin A, that terminates in a C-terminal CAAX motif (where C is cysteine, A is generally an aliphatic amino acid, and X is any residue). Like all CAAX proteins, prelamin A undergoes three sequential posttranslational modifications including isoprenylation of cysteine with a farnesyl lipid moiety, endoproteolytic removal of the -AAX peptide by ZMPSTE24 (or by RCE1), and carboxyl methylation (*1*, *2*) (see the figure). Unlike most other CAAX proteins, however, prelamin A undergoes a second cleavage event, also mediated by ZMPSTE24, to yield mature lamin A. This second cleavage removes the last 15 amino acids of the protein, including the newly modified C terminus (*4*, *7*–*9*). The

[1]Department of Cell Biology, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA. [2]Department of Chemistry, Purdue University, West Lafayette, IN 47907, USA. E-mail: michaelis@jhmi.edu; hrycyna@purdue.edu



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In the hollow. (**A**) In the posttranslational modification of lamin A, the zinc metalloprotease ZMPSTE24 (or RCE1) mediates processing of the -CAAX motif; ZMPSTE24 then makes a second cleavage. (**B**) ZMPSTE24 is a membrane protein with a large hollow barrel-shaped chamber enclosing the active site. In the hypothetical reaction scheme shown, the C terminus of lamin A (blue), which has been modified with a farnesyl moiety (green line), enters the cavity through a gap in the chamber wall between two transmembrane spans, and aligns in the Zn^{2+} active site. ZMPSTE24 removes the -AAX peptide. The membrane protein ICMT then methylates (Me) lamin A. This is followed by removal of the modified C terminus by ZMPSTE24 and the release of lamin A.

ZMPSTE24 structure reported by Quigley *et al*. sets the stage for determining how this protease recognizes two completely different cleavage sites within lamin A.

Although it remains a mystery as to why cells go to the trouble of modifying the C-terminus of lamin A only to have ZMPSTE24 remove it, the finding highlights its role in aging disorders and suggests a potentially important role for the metalloprotease in normal human aging. It will be critical to ensure that new protease inhibitors and other drug types do not inadvertently block ZMPSTE24 through unintended off-target interactions. Already, an unexpected in vitro inhibition of ZMPSTE24 by several HIV protease inhibitors has been shown that could possibly contribute to the side effects of HIV treatment (*10*).

The most surprising result from the essentially superimposable crystal structures of human ZMPSTE24 and yeast Ste24p is the presence of a voluminous membrane-enclosed hollow chamber, not previously seen in a membrane protein, which could serve as an enzymatic reaction vessel. This barrel-shaped chamber, formed by seven transmembrane segments and capped at both ends, is estimated by Pryor *et al*. to be large enough to accommodate ~450 water molecules or a ~10-kD protein. The structures show that the active site of these zinc metalloproteases faces the interior of the chamber at the membrane interface. Thus, after a prenylated substrate enters the chamber, it is ideally poised for proteolysis. Interestingly, the structures illustrate how several MAD-B and RD disease mutations that block enzymatic activity (*11*) could affect the zinc metalloprotease active site or access to it.

The voluminous hollow chamber is far larger than needed simply for catalysis, presenting a challenging puzzle as to its function. One possibility is that the chamber sequesters the farnesylated 15–amino acid lamin A tail generated in the final proteolytic step of prelamin A processing. Persistent farnesylation and methylation of prelamin A is implicated in disease states. Thus, it is conceivable that the cleaved peptide itself may confer similar phenotypes if allowed to freely diffuse within the membrane. Sequestration and possibly further proteolysis of the peptide in the chamber might neutralize such potentially toxic effects.

The ZMPSTE24 and Ste24p structures also begin to provide clues as to how substrates undergo dual cleavage. The C-terminal farnesylated portion of the substrate could insert in the active-site cavity through a gap in the chamber wall between two transmembrane helices. After proteolysis of the -AAX sequence and prior to the second cleavage, the farnesylated cysteine on the substrate is methylated by isoprenylcysteine carboxyl methyltransferase (ICMT). It has been assumed that ZMPSTE24/Ste24p releases the substrate and then rebinds it after methylation. However, the structures raise the intriguing possibility of a processive mechanism, in which the substrate never completely leaves the chamber. In this model, after the first cleavage, the substrate would move through the enzyme, project outward through another gap between membrane spans, undergo methylation by ICMT (itself an integral membrane enzyme), and then retract back into the chamber for the final cleavage. Regardless of the mechanism, the roles of the farnesyl and methyl groups in positioning the substrate for the cleavage by ZMPSTE24/Ste24p remain an open question.

Ultimately, determining how ZMPSTE24 functions and how to avoid accidentally interfering with its function will have important implications for pharmaceutical drug design. This knowledge will also widen our understanding of premature aging diseases and normal physiological aging.

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CHEMISTRY

FRETting over the Spectroscopic Ruler

Time-resolved ultraviolet spectra of myoglobin show that electron transfer can compete with Förster resonance energy transfer at short distances.

Jay R. Winkler

Beckman Institute, California Institute of Technology, 1200 East California Boulevard, Pasadena, CA 91125, USA. E-mail: winklerj@caltech.edu

An excited fluorescent molecule may not emit light if it can transfer its excitation energy to a nearby molecule. Förster's theory describing electronic excitation energy transfer (EET) (*1*) is now 65 years old, but it is not ready for retirement. It still enjoys popularity through the FRET (*2*) method for determining molecular-scale distances. A seminal paper by Stryer and Haugland in 1967 provided experimental support for the predicted inverse sixth power distance dependence of FRET efficiency and coined the term "spectroscopic ruler" (*2*). Despite the widespread use of fluorescence spectroscopy to determine distances between a "donor" (D) and "acceptor" (A), the method can be tricky, and numerous caveats concerning the FRET model have appeared. On page 1586 of this issue, Consani *et al*. (*3*) point out another, based on their use of two-dimensional ultraviolet (UV) transient spectroscopy to disentangle the competing contributions of electron transfer and FRET to the decay of excited tryptophan (Trp) residues in myoglobin (Mb).

Förster's elegant derivation (*1*) began with a golden rule expression for the FRET transition rate. The electronic matrix element described the Coulombic interaction between electrons on D and A. In the limit where D and A are separated by a distance R_{DA} that is substantially greater than the dimensions of D and A, the Coulombic potential can be approximated by the inter-

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