McVean, G.A., Gabriel, S.B., Reich, D., Donnelly, P., *et al*. (2005). Comparison of fine-scale recombination rates in humans and chimpanzees. Science *308*, 107–111.
14. Ptak, S.E., Hinds, D.A., Koehler, K., Nickel, B., Patil, N., Ballinger, D.G., Przeworski, M., Frazer, K.A., and Paabo, S. (2005). Fine-scale recombination patterns differ between chimpanzees and humans. Nat. Genet. *37*, 429–434.
15. Mihola, O., Trachtulec, Z., Vlcek, C., Schimenti, J.C., and Forejt, J. (2009). A mouse speciation gene encodes a meiotic histone H3 methyltransferase. Science *323*, 373–375.

[1]Whitehead Institute for Biomedical Research, Cambridge, MA 02142, USA.
[2]Université de Lyon, Université Lyon 1, Centre National de la Recherche Scientifique, UMR5558, Laboratoire de Biométrie et Biologie évolutive, Villeurbanne, F-69622 cedex, France.
E-mail: andi@wi.mit.edu, marais@biomserv.univ-lyon1.fr

DOI: 10.1016/j.cub.2010.01.048

Mitochondrial Function: OMA1 and OPA1, the Grandmasters of Mitochondrial Health

Two new studies have identified a key protease responsible for sensing mitochondrial dysfunction, leading to the inactivation of the fusion GTPase OPA1. These studies have broad implications in mitochondrial quality control.

Heidi McBride and Vincent Soubannier

The regulation of mitochondrial fusion has recently emerged as a critical factor in the exclusion of respiration-incompetent organelles, suggesting that the balance of fission and fusion is central to mitochondrial quality control [1]. Fragments of mitochondria that cannot generate or maintain their electrochemical potential become fusion-incompetent and are degraded through autophagy [1]. Mechanistically, it has been shown that uncoupling the mitochondrial membrane potential ($\Delta\Psi$) leads to the cleavage of the GTPase OPA1, which is localised to the intermembrane space and is required for mitochondrial fusion [2–4]. This sounds rather simple, yet the story of OPA1 processing has been complicated, with many candidate proteases proposed to participate in this regulated OPA1 cleavage. Two recent studies published in the *Journal of Cell Biology* have resolved this issue by identifying a novel zinc metalloprotease called OMA1 as the essential $\Delta\Psi$-dependent protease for OPA1 [5,6].

OPA1 is alternatively spliced, giving rise to eight variants that are expressed in a variety of patterns depending on the tissue [7]. The different splice variants possess either one or two cleavage sites, called S1 and S2 [3,4]. Previous work has shown that OPA1 must be present in both long and short forms for fusion to proceed, with the balance of those forms being maintained by constitutive processing [8]. The S2 site is a substrate for the intermembrane space AAA (i-AAA) protease YME1L, and it is generally agreed that this protease cleaves OPA1 constitutively following mitochondrial import [3,4] (Figure 1A). Although there is some constitutive cleavage at the S1 site, which is common to all variants, this site is the primary target of regulated cleavage upon loss of $\Delta\Psi$ [3,4]. In this case, or under conditions of low mitochondrial ATP levels or the presence of apoptotic signals, all long forms become cleaved at site S1, thereby disrupting the balance of long and short forms, abolishing mitochondrial fusion. The field has been searching for a convincing protease that mediates this regulated cleavage. A number of studies have suggested that various proteases, including PARL, paraplegin, and the AFG3L1/AFG3L1 mAAA protease complex, may be partly responsible [8].

The uncertainty surrounding the protease that mediates regulated OPA1 cleavage has now been resolved with the new papers characterising the mammalian inner membrane protease OMA1. These two groups have independently discovered that the loss of OMA1 through silencing completely abolishes the regulated cleavage of OPA1 [5,6]. In this way, the two new publications make it clear that OMA1 acts as a primary determinant of fusion competence. Mechanistically, however, the two studies focus on different aspects of how OMA1 activity may be regulated, and in this respect many questions are raised.

OMA1 is a zinc metalloprotease that spans the inner mitochondrial membrane with a number of predicted membrane spanning domains [9]. It has been most well characterized in yeast where it has overlapping activity with the matrix AAA (m-AAA) proteases (hence the name OMA1) [9]. The human orthologue of OMA1 (called MPRP-1) was originally mistakenly localized to the endoplasmic reticulum, where it went unnoticed since 2003 [10]. The current studies clearly demonstrate an exclusively mitochondrial localization of OMA1 [5,6]. The mammalian orthologue described in these two new studies has evolved a long 170 amino acid extension at the amino terminus that may impact the topology in higher organisms [10]. It will be essential to establish the topology of this multispanning membrane protein biochemically, and to localize the catalytic site in order to better understand its regulation.

The role of the amino-terminal extension is highlighted in the study by Head *et al.* [5], where they show that the 60 kDa precursor form of OMA1 is cleaved upon mitochondrial import to a 40 kDa mature form by an as yet unidentified protease. Upon dissipation of $\Delta\Psi$, the 60 kDa form of OMA1 rapidly accumulated concomitantly with the cleavage of all OPA1 variants, prompting the authors to consider that the 40 kDa form of OMA1 may be the inactive form, with the 60 kDa form being the active enzyme. In this way, the ongoing import of newly synthesized OMA1 into the inner membrane upon collapse of $\Delta\Psi$ would facilitate active cleavage of OPA1 at the S1 site. Although it is possible to generate mutants or chimeras of model proteins whose import into the inner membrane may become less sensitive to dissipation of $\Delta\Psi$, there are currently no known

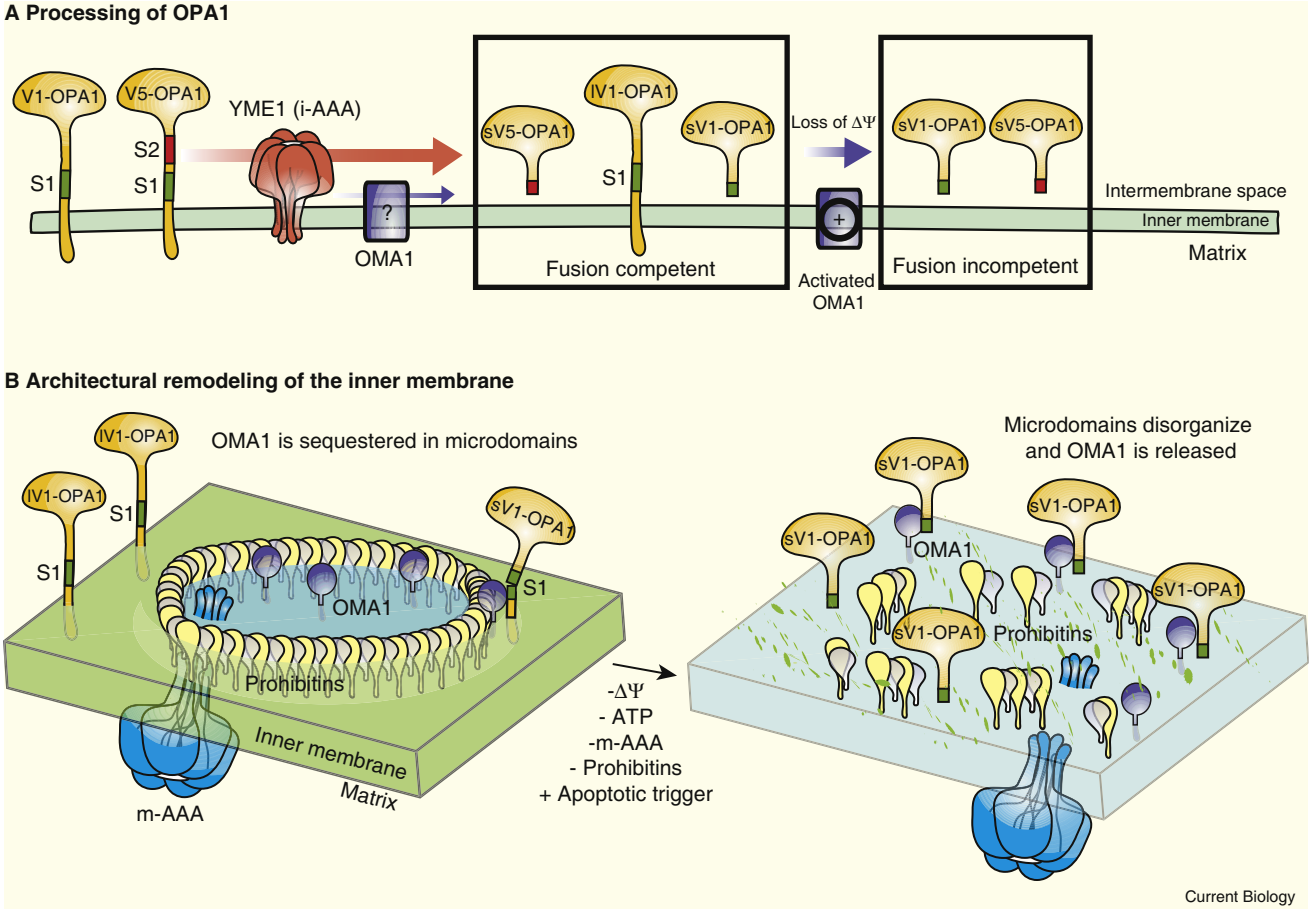

Figure 1. Models of OPA1 processing and OMA1 activation.

(A) Following mitochondrial import, short OPA1 isoforms are produced via the processing at site S2 by the i-AAA protease YME1L. Other short isoforms may be obtained by the cleavage at site S1, which is regulated by the tightly controlled OMA1 activity. Under conditions of mitochondrial dysfunction, OMA1 activity is upregulated, resulting in the cleavage of all OPA1 isoforms at site S1, which results in inhibition of mitochondrial fusion. (B) The different signals activating OPA1 could be integrated through the existence of a functional platform. Rings of prohibitins, associated with m-AAA proteases, would form a microdomain sequestering OMA1 from its substrates. Mitochondrial dysfunction would lead to the destabilization and/or disassembly of these platforms, releasing OMA1 to access its substrates in the rest of the inner membrane.

examples of endogenous protein import into the inner membrane that would be $\Delta\Psi$ independent [11]. Instead, the data presented by Head *et al.* [5] show the import of a 60 kDa precursor protein that is carbonate resistant, indicating membrane integration, and protease accessible, suggesting exposure to the cytosol. From this, we consider that the simplest interpretation of these data is that, upon mitochondrial dysfunction, the newly translated OMA1 may insert into the outer membrane, exposing the protease domain in the intermembrane space to cleave OPA1. Although this model is derived from the data presented by Head *et al.* [5], the idea that polytopic membrane proteins may be differentially imported into the two mitochondrial membranes is obviously unprecedented and a great deal of experimentation is required to test this idea.

Additional mechanistic data were presented by the accompanying paper from the Langer group [6], focusing on the role of the ATP-dependent m-AAA metalloproteases in regulating the activity of OMA1. Given that the presentation of these proteases to OPA1 in the yeast model system led to the processing of OPA1 from the long form into the short form [12], the authors tested this potential function in the mammalian system. Surprisingly, their data reveal an opposite phenotype, in which the loss of the proteases instead activated the cleavage of OPA1 at the S1 site [6]. These data clearly indicate that the m-AAA proteases, although capable of cleaving OPA1 in a heterologous system, appear to negatively regulate OPA1 cleavage at the S1 site in mammalian cells. Importantly, although the m-AAA proteases have been linked to mitochondrial quality control [13], the authors show that mitochondria lacking the m-AAA proteases under normal conditions are metabolically healthy. Therefore, the authors ascribe a direct function for the m-AAA proteases in the regulation of OPA1 cleavage, rather than an indirect effect due to global mitochondrial dysfunction.

To identify the protease activated upon loss of the m-AAA proteases, they looked to OMA1, shown in yeast to have overlapping functions with the m-AAA proteases [9]. Indeed, silencing OMA1 in cells lacking m-AAA proteases reversed their phenotypes and no OPA1 cleavage was observed [6]. So why does the loss of m-AAA proteases

lead to the activation of OMA1 protease activity towards OPA1? Given previous work from the Langer lab, one model to potentially explain the results of Ehses *et al.* [6] would invoke the involvement of inner membrane lipid microdomains [14]. Prohibitins are inner membrane proteins thought to possess chaperone-like activity. These proteins are found in very large, megadalton complexes thought to function in the initiation of lipid microdomains, favouring the local assembly of functional platforms [15]. These functional platforms, which are enriched with prohibitin-binding partners, such as the m-AAA proteases, may sequester OMA1 to ensure its rapid cleavage and control the access of OMA1 to its substrates. Indeed, the bacterial homologues of OMA1 are highly catalytically active [16], suggesting that OMA1 must be kept under tight, yet reversible, control. It is then conceivable that the loss of the m-AAA proteases may disrupt these platforms, thereby allowing OMA1 to become available to process OPA1 (Figure 1B). This would couple inner membrane disorganization with the inhibition of mitochondrial fusion, resulting in the elimination of the fragmented, disorganized organelle.

Finally, the role of OMA1 in the regulated cleavage of OPA1 is an evolutionary twist on the simpler system in yeast for the cleavage of the OPA1 homologue Mgm1. In yeast, the rhomboid protease Rbd1/Pcp1 cleaves Mgm1 under conditions of high energy [17]. Under low ATP conditions, Mgm1 cannot be pulled across the import channel by the matrix chaperones and instead its translocation is arrested at the first hydrophobic domain, resulting in the accumulation of the long, uncleaved forms of Mgm1, thereby inactivating fusion. Rather than altering the topology of OPA1, it appears that the mammalian mitochondria survey their health through the altered topology and activity of the OMA1 protease. The integration of OMA1 into OPA1 cleavage appears to be missing in *Drosophila melanogaster* or *Caenorhabditis elegans*, suggesting that these organisms may regulate OPA1 processing in a manner homologous to yeast. Indeed, work in flies has shown a role for the rhomboid protease Rhomboid-7 in OPA1 cleavage [18]. It will be important in future work to uncover the functional implications for these different mechanistic pathways that regulate OPA1/Mgm1 cleavage.

References

1. Mouli, P.K., Twig, G., and Shirihai, O.S. (2009). Frequency and selectivity of mitochondrial fusion are key to its quality maintenance function. Biophys. J. *96*, 3509–3518.
2. Duvezin-Caubet, S., Jagasia, R., Wagener, J., Hofmann, S., Trifunovic, A., Hansson, A., Chomyn, A., Bauer, M.F., Attardi, G., Larsson, N.G., *et al*. (2006). Proteolytic processing of OPA1 links mitochondrial dysfunction to alterations in mitochondrial morphology. J. Biol. Chem. *281*, 37972–37979.
3. Griparic, L., Kanazawa, T., and van der Bliek, A.M. (2007). Regulation of the mitochondrial dynamin-like protein Opa1 by proteolytic cleavage. J. Cell Biol. *178*, 757–764.
4. Song, Z., Chen, H., Fiket, M., Alexander, C., and Chan, D.C. (2007). OPA1 processing controls mitochondrial fusion and is regulated by mRNA splicing, membrane potential, and Yme1L. J. Cell Biol. *178*, 749–755.
5. Head, B., Griparic, L., Amiri, M., Gandre-Babbe, S., and van der Bliek, A.M. (2009). Inducible proteolytic inactivation of OPA1 mediated by the OMA1 protease in mammalian cells. J. Cell Biol. *187*, 959–966.
6. Ehses, S., Raschke, I., Mancuso, G., Bernacchia, A., Geimer, S., Tondera, D., Martinou, J.C., Westermann, B., Rugarli, E.I., and Langer, T. (2009). Regulation of OPA1 processing and mitochondrial fusion by m-AAA protease isoenzymes and OMA1. J. Cell Biol. *187*, 1023–1036.
7. Delettre, C., Griffoin, J.M., Kaplan, J., Dollfus, H., Lorenz, B., Faivre, L., Lenaers, G., Belenguer, P., and Hamel, C.P. (2001). Mutation spectrum and splicing variants in the OPA1 gene. Hum. Genet. *109*, 584–591.
8. Soubannier, V., and McBride, H.M. (2009). Positioning mitochondrial plasticity within cellular signaling cascades. Biochim. Biophys. Acta *1793*, 154–170.
9. Kaser, M., Kambacheld, M., Kisters-Woike, B., and Langer, T. (2003). Oma1, a novel membrane-bound metallopeptidase in mitochondria with activities overlapping with the m-AAA protease. J. Biol. Chem. *278*, 46414–46423.
10. Bao, Y.C., Tsuruga, H., Hirai, M., Yasuda, K., Yokoi, N., Kitamura, T., and Kumagai, H. (2003). Identification of a human cDNA sequence which encodes a novel membrane-associated protein containing a zinc metalloprotease motif. DNA Res. *10*, 123–128.
11. Geissler, A., Krimmer, T., Bomer, U., Guiard, B., Rassow, J., and Pfanner, N. (2000). Membrane potential-driven protein import into mitochondria. The sorting sequence of cytochrome b(2) modulates the deltapsi-dependence of translocation of the matrix-targeting sequence. Mol. Biol. Cell *11*, 3977–3991.
12. Duvezin-Caubet, S., Koppen, M., Wagener, J., Zick, M., Israel, L., Bernacchia, A., Jagasia, R., Rugarli, E.I., Imhof, A., Neupert, W., *et al*. (2007). OPA1 processing reconstituted in yeast depends on the subunit composition of the m-AAA protease in mitochondria. Mol. Biol. Cell *18*, 3582–3590.
13. Tatsuta, T., and Langer, T. (2009). AAA proteases in mitochondria: diverse functions of membrane-bound proteolytic machines. Res. Microbiol. *160*, 711–717.
14. Osman, C., Merkwirth, C., and Langer, T. (2009). Prohibitins and the functional compartmentalization of mitochondrial membranes. J. Cell Sci. *122*, 3823–3830.
15. Osman, C., Haag, M., Potting, C., Rodenfels, J., Dip, P.V., Wieland, F.T., Brugger, B., Westermann, B., and Langer, T. (2009). The genetic interactome of prohibitins: coordinated control of cardiolipin and phosphatidylethanolamine by conserved regulators in mitochondria. J. Cell Biol. *184*, 583–596.
16. Sakoh, M., Ito, K., and Akiyama, Y. (2005). Proteolytic activity of HtpX, a membrane-bound and stress-controlled protease from Escherichia coli. J. Biol. Chem. *280*, 33305–33310.
17. Herlan, M., Bornhovd, C., Hell, K., Neupert, W., and Reichert, A.S. (2004). Alternative topogenesis of Mgm1 and mitochondrial morphology depend on ATP and a functional import motor. J. Cell Biol. *165*, 167–173.
18. McQuibban, G.A., Lee, J.R., Zheng, L., Juusola, M., and Freeman, M. (2006). Normal mitochondrial dynamics requires rhomboid-7 and affects Drosophila lifespan and neuronal function. Curr. Biol. *16*, 982–989.

University of Ottawa Heart Institute,
40 Ruskin Street, Ottawa,
Ontario K1Y 4W7, Canada.
E-mail: hmcbride@ottawaheart.ca

DOI: 10.1016/j.cub.2010.02.011

Behavioral Neurobiology: Leech Lust in the Lab

Animals typically will not exhibit reproductive behaviors during invasive experimental manipulations. The demonstration of courtship-related impulse activity in isolated leech ganglia promises new opportunities to elucidate the neural basis of mating.

Kevin M. Crisp

Charles Darwin suggested that one sign of the intelligence of earthworms is their apparent obliviousness to their surroundings during copulation. “Their sexual passion,” he wrote, “is strong enough to over come for a time their dread of light”, which they avoid instinctively at other times [1]. This single-minded focus during mating is not characteristic of most animals. Stereotypic courtship and mating behaviors are rarely elicited from