interactions. Specifically, CASK is known to interact with ZO1, which interacts with JAM2, which interacts with Pard3, thus bringing CASK and Pard3 into proximity. However, the work presented here shows that CASK and Pard3 interact directly through dimerization of their PDZ domains. Why these two proteins interact directly requires further investigation, but now that Chang et al. (2011) have identified every PDZ-PDZ interaction from the mouse proteome, they have paved the way for establishing the biological significance of these interactions.

REFERENCES

Chang, B.H., Gujral, T.S., Karp, E.S., BuKhalid, R., Grantcharova, V.P., and MacBeath, G. (2011). Chem. Biol. *18*, this issue, 1143–1152.

Hartwell, L.H., Hopfield, J.J., Leibler, S., and Murray, A.W. (1999). Nature *402* (6761, Suppl), C47–C52.

Heyduk, T., Ma, Y., Tang, H., and Ebright, R.H. (1996). Methods Enzymol. *274*, 492–503.

Hillier, B.J., Christopherson, K.S., Prehoda, K.E., Bredt, D.S., and Lim, W.A. (1999). Science *284*, 812–815.

MacBeath, G., and Schreiber, S.L. (2000). Science *289*, 1760–1763.

Oda, K., Matsuoka, Y., Funahashi, A., and Kitano, H. (2005). Mol. Syst. Biol. *1*, 2005.0010.

Songyang, Z., Fanning, A.S., Fu, C., Xu, J., Marfatia, S.M., Chishti, A.H., Crompton, A., Chan, A.C., Anderson, J.M., and Cantley, L.C. (1997). Science *275*, 73–77.

Chemical Activators of ClpP: Turning Jekyll into Hyde

David A. Dougan[1,*]
[1]Department of Biochemistry, La Trobe Institute for Molecular Science, La Trobe University, Melbourne 3086, Australia
*Correspondence: d.dougan@latrobe.edu.au
DOI 10.1016/j.chembiol.2011.09.003

Casein lytic peptidase P (ClpP) is a serine peptidase that, when coupled to its cognate ATPase, facilitates the controlled degradation of both damaged and unwanted proteins in bacteria. In this issue of *Chemistry & Biology*, Leung et al. (2011) report a small molecule screen against ClpP, from which they identified four structurally distinct compounds that activate ClpP for unregulated proteolysis.

The treatment of bacterial infections with antimicrobial drugs was one of the most profound medical advances of the last century. The discovery of these drugs began in the 1930s and continued unabated over the next four decades. Indeed, many of the drugs we use today can be traced back to natural compounds, identified during these "golden" years of drug discovery, and their effectiveness is evidenced by our current quality of life. However, since the end of this fruitful period of drug discovery, relatively few new compounds (natural or synthetic) have been developed. Concomitantly, especially during the last decade, there has been a concerning increase in the occurrence of nosocomial infections involving drug resistant bacterial species (e.g., Methicillin-resistant *Staphylococcus aureus* [MRSA] and Vancomycin-resistant *Enterococci* [VRE]) (Levy and Marshall, 2004) that has in turn lead to the emergence of multi-drug resistant (MDR) bacteria. Hence, there is a real need for the development of new drugs, especially those that target novel mechanisms to kill bacterial cells.

In 2005, Brötz-Oesterhelt and colleagues identified a new class of natural antibiotics termed acyldepsipeptides (ADEPs) that showed remarkable promise, as they were active in the treatment of rodents infected with antibiotic resistant bacteria (Brötz-Oesterhelt et al., 2005). Surprisingly, these compounds do not kill bacteria by inhibiting an essential cellular process, but rather they target a non essential protein, the peptidase ClpP, to kill bacteria. Indeed ADEPs are proposed to kill bacteria via a unique mechanism—by triggering the widespread and unregulated degradation of nascent polypeptides and unfolded proteins (Kirstein et al., 2009). Despite their remarkable bactericidal activity, limited availability of these antibiotics has hampered progress in elucidating their mechanism of action; hence the identification of new ClpP activators of unregulated proteolysis may aid in further defining how this promising class of drug functions.

ClpP is a barrel-shaped protein composed of two heptameric rings in which the catalytic residues are sequestered inside a proteolytic chamber. In the absence of its cognate AAA+ (ATPase associated with various cellular activities) component (e.g., ClpA, ClpC or ClpX), entry into this chamber is restricted to a narrow entry portal at either end of the complex (Wang et al., 1997). In this state, although short peptides can enter the proteolytic chamber for hydrolysis, large polypeptides are generally excluded from the chamber, preventing the indiscriminate degradation of cellular proteins. Therefore, in the absence of its cognate ATPase, protein degradation by ClpP is effectively turned OFF (Figure 1). By contrast, in the presence of its cognate ATPase, ClpP-mediated protein degradation is turned ON (Figure 1). Currently, it is widely accepted that activation of ClpP results from docking of a specific loop (known as the IGF loop) on the cognate ATPase (Kim et al., 2001), which culminates in opening of the narrow entry portal located at the distal ends of the complex, supporting entry of unfolded polypeptides, into the proteolytic chamber (Burton et al., 2001).

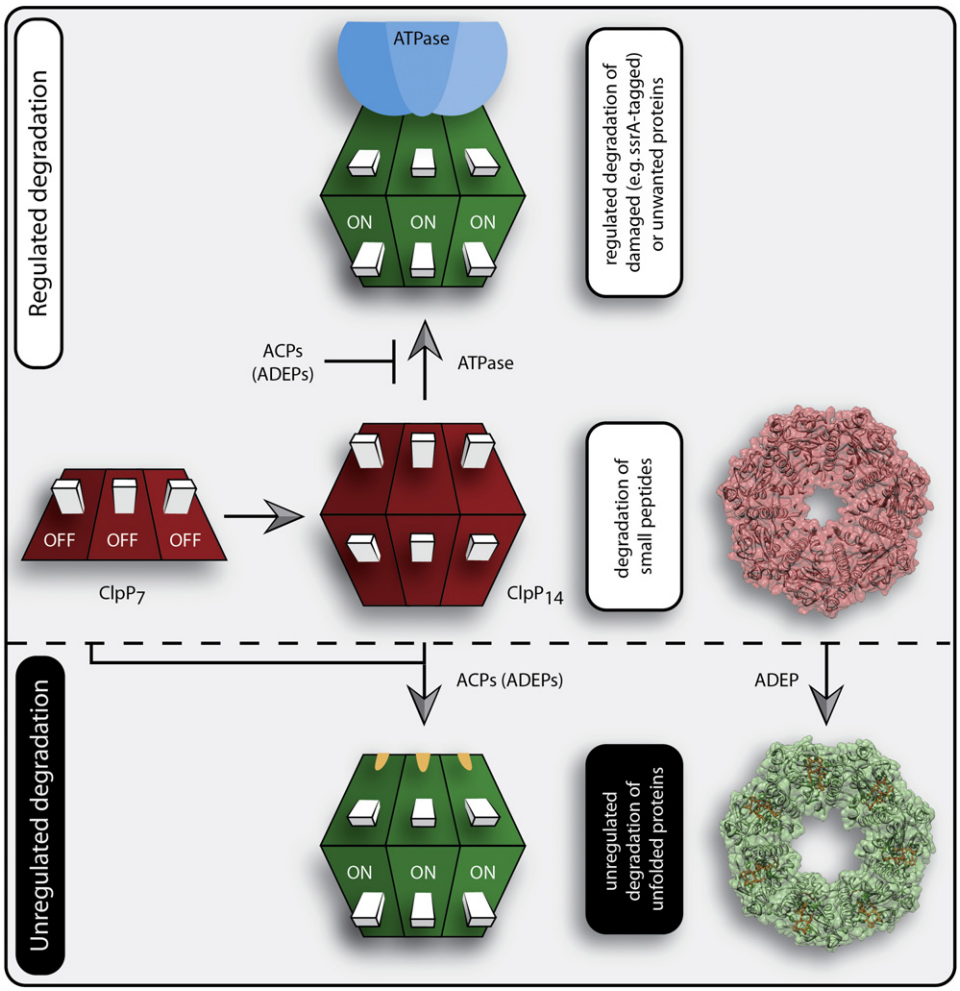

Figure 1. Activators of Self-Compartmentalizing Proteases Turn ClpP On for "Unregulated" Proteolysis
ClpP-mediated protein degradation is normally a highly regulated process in bacteria. Under normal conditions, the degradation of large protein substrates by single- or double-ringed oligomers of ClpP is effectively turned OFF (red) and only small peptides can access the catalytic residues located inside the proteolytic chamber. Consistently, the pore of ClpP is "closed," diameter of ~10 Å (red ring, left side). However, in the presence of its cognate ATPase (blue), ClpP is turned ON (green) and the regulated degradation of selected substrates (e.g., SsrA-tagged proteins) can proceed. The SsrA tag is an 11 amino acid motif, attached to the C terminus of "stalled" translation products in bacteria, that is essential for ATPase-mediated recognition of these incomplete proteins. By contrast, ACPs and ADEPs (yellow) activate ClpP for unregulated protein degradation by opening the pore to a diameter ~20 Å (green ring, right side), permitting the entry of large unfolded proteins into the catalytic chamber of ClpP in the absence of its cognate ATPase component.

In the current study, Leung et al. (2011) exploit the idea that ClpP is a useful drug target and have developed a simple fluorescence-based assay to identify small molecules that activate ClpP for ATPase-independent or "unregulated" degradation of large polypeptides. Specifically, they employed a high-throughput screen to monitor the ClpP-mediated degradation of a fluorescently labeled model "unfolded" protein (Fluorescein isothiocyanate labeled casein or FITC-casein). In the absence of ClpP activation, FITC-casein is stable, and hence the fluorescence signal is quenched; however upon activation of ClpP, FITC-casein is degraded into short FITC-labeled peptides, which are highly fluorescent. Using this assay, the authors screened approximately 60,000 small molecules from which they identified five new compounds (belonging to four different structural classes), called activators of self-compartmentalizing proteases (ACPs; ACP1–ACP5). Importantly, all five compounds were able to trigger the nonspecific degradation of several "unfolded" proteins; however, some compounds were considered better ClpP activators than others, as they were able to trigger the degradation of a broader range of substrates. From these five compounds, the best activator of ClpP - ACP1 (which was also considered to display good drug-like characteristics) was chosen for optimization and over 70 new derivatives were created, one of which (ACP1b) exhibited dramatically improved binding characteristics and activation qualities when compared to the parent compound.

Using a variety of biophysical techniques, the authors revealed that ACPs, like their natural cousins (ADEPs), activate ClpP through stabilization of the tetradecamer (Figure 1). Indeed, there was a good correlation between the binding affinity of the compound, stabilization of the double-ringed oligomer and activation of ClpP.

Also, through a combination of molecular docking simulations and biochemical experiments using various ClpP mutant proteins, the authors proposed that ACPs target two pockets on the surface of ClpP. Strong ACPs (e.g., ACP1b) bind exclusively to a hydrophobic pocket (H-pocket) located on the apical surface of ClpP, while weak ACPs (e.g., ACP2) appear to bind to both the H-pocket and a second pocket called the C-pocket (as it contains several charged residues), which is formed by the interface of two adjacent subunits in the heptameric ring. Consistent with an important role for the H-pocket in ClpP activation, recent structural analysis of ClpP in complex with ADEP (Lee et al., 2010; Li et al., 2010) revealed that ADEPs dock into the H-pocket, causing a conformational change in ClpP that triggers a dramatic opening of the entry portal into the proteolytic chamber (see Figure 1, right side). Interestingly, the ClpP activators (that bind exclusively to the H-pocket) not only trigger unregulated degradation of unfolded proteins in the absence of the cognate ATPase, but also inhibit the tightly regulated degradation of a model SsrA-tagged protein (i.e., GFP-ssrA), which represents an ATPase-dependent substrate. Collectively, these findings suggest that chemical activators of ClpP, such as ACPs and ADEPs, may function as IGF-loop mimetics, and hence these compounds may prove useful, not only in the development of future antimicrobial drugs but also in providing useful mechanistic insights into how ATPase(s) activate ClpP for degradation. Indeed this work reinforces the utility of ClpP as a potential drug target and provides a number of new structural frameworks from which potentially useful ClpP activators could be further developed into antibacterial drugs. Nevertheless, given that ClpP is also expressed in mammalian mitochondria where it is proposed to play an important role in the mitochondrial specific unfolded protein response, one may need to be cautious about the use of antibacterial drugs that target ClpP until the effect of these drugs has been studied on human ClpP and mitochondrial function. Alternatively, drugs that target HslV (a bacterial self-compartmentalized protease that is absent in higher eukaryotes) for unregulated degradation may have great promise.

ACKNOWLEDGMENTS

The author wishes to thank Kursad Turgay and Kaye Truscott for their helpful comments on the manuscript. D.A.D. is an Australian Research Fellow and work in his laboratory is supported by the Australian Research Council.

REFERENCES

Brötz-Oesterhelt, H., Beyer, D., Kroll, H.P., Endermann, R., Ladel, C., Schroeder, W., Hinzen, B., Raddatz, S., Paulsen, H., Henninger, K., et al. (2005). Nat. Med. *11*, 1082–1087.

Burton, R.E., Siddiqui, S.M., Kim, Y.I., Baker, T.A., and Sauer, R.T. (2001). EMBO J. *20*, 3092–3100.

Kim, Y.I., Levchenko, I., Fraczkowska, K., Woodruff, R.V., Sauer, R.T., and Baker, T.A. (2001). Nat. Struct. Biol. *8*, 230–233.

Kirstein, J., Hoffmann, A., Lilie, H., Schmidt, R., Rubsamen-Waigmann, H., Brotz-Oesterhelt, H., Mogk, A., and Turgay, K. (2009). Mol. Med. *1*, 37–49.

Lee, B.G., Park, E.Y., Lee, K.E., Jeon, H., Sung, K.H., Paulsen, H., Rübsamen-Schaeff, H., Brötz-Oesterhelt, H., and Song, H.K. (2010). Nat. Struct. Mol. Biol. *17*, 471–478.

Leung, E., Datti, A., Cossette, M., Goodreid, J., McCaw, S.E., Mah, M., Nakhamchik, A., Ogata, K., El Bakkouri, M., Cheng, Y.-Q., et al. (2011). Chem. Biol. *18*, this issue, 1167–1178.

Levy, S.B., and Marshall, B. (2004). Nat. Med. ***10*** (12, Suppl), S122–S129.

Li, D.H., Chung, Y.S., Gloyd, M., Joseph, E., Ghirlando, R., Wright, G.D., Cheng, Y.Q., Maurizi, M.R., Guarné, A., and Ortega, J. (2010). Chem. Biol. *17*, 959–969.

Wang, J., Hartling, J.A., and Flanagan, J.M. (1997). Cell *91*, 447–456.

