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CELLULAR MICROBIOLOGY

Tagging proteins for the Clp protease

> arginine phosphorylation tags proteins for degradation by the ClpCP protease

Bacterial Clp proteases degrade proteins in all bacteria, but whether they recognize a specific 'tag' on their targets, similar to the mechanism in eukaryotic cells in which ubiquitin tags proteins for proteasome-mediated degradation, is unclear. Now, Trentini, Suskiewicz *et al.* reveal that arginine phosphorylation targets proteins for degradation by the ClpCP protease that is produced by *Bacillus subtilis*.

The ATP-dependent protease ClpCP comprises the AAA+ unfoldase ClpC and the protease ClpP, and it is regulated by the arginine kinase McsB. For example, McsB increases the amount of ClpCP in cells and acts as an adaptor protein to promote the ATPase activity of ClpC. In this study the authors found that 14 proteins that were phosphorylated on arginine (pArg) were pulled down by an engineered ClpP 'cage' in wild-type *B. subtilis*, whereas only one pArg protein was pulled down by this ClpP cage in *clpC*-knockout cells. This indicates that ClpC might direct pArg proteins for ClpP-mediated proteolysis. In experiments that used the intrinsically unfolded protein β-casein as a protease target, McsB induced the ClpCP-mediated degradation of β-casein; this degradation was inhibited by the addition of an arginine phosphatase and through the inactivation of the kinase activity of McsB. Thus, McsB-mediated arginine phosphorylation is required for ClpCP-mediated protein degradation.

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A series of experiments further confirmed that pArg tags targets for ClpCP. β-casein that was phosphorylated on arginine (pArg–β-casein), but not unphosphorylated β-casein, could interact with the amino-terminal domain (NTD) of ClpC. Furthermore, ClpCP could degrade pArg–β-casein in the absence of McsB, which suggests that this protease complex can form without an adaptor; this was confirmed by pull-down experiments that showed that pArg–β-casein, but not unphosphorylated β-casein, could promote ClpCP assembly. Finally, degradation assays that involved β-casein proteins that were phosphorylated on arginine to different extents revealed that the degree of ClpCP-mediated degradation positively correlated with the extent of β-casein phosphorylation. Thus, ClpCP specifically degrades pArg–β-casein.

With the aim to reveal how pArg binds to ClpC, the authors determined the crystal structure of the NTD from *B. subtilis* ClpC together with the free amino acid phosphoarginine ($pArg^{AA}$). The structure, at a resolution of 1.6 Å, revealed that the NTD of ClpC has two pArg binding sites. These sites are almost identical and seem to interact with pArg through Glu32 and Glu106. The role of these glutamate residues in pArg–ClpC interactions was confirmed by data that showed that the NTD domain of ClpC in which they were mutated could not bind to $pArg^{AA}$, or be activated by pArg–β-casein, unless incubated in the presence of another ClpC adaptor protein, MecA. Furthermore, the expression of wild-type ClpC, but not of ClpC in which these glutamates were mutated, could suppress growth defects of *clpC*-null *B. subtilis* grown at increased temperatures, which highlights the biological importance of pArg in ClpC-mediated protein degradation. Finally, although the structural data indicate that ClpC contains 12 sites to which pArg can bind, experimental data led the authors to propose that ClpC targets can be tagged for degradation by just one pArg.

In summary, this study reveals that pArg tags proteins for ClpCP-mediated degradation in *B. subtilis*. Given how the discovery of ubiquitin changed the landscape of protein research in eukaryotes, and the fact that Glu32 and Glu106 were found to be conserved in ClpC proteins from other Gram-positive bacteria, this discovery is likely to open new avenues of investigation for understanding protein turnover in bacteria.

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