BACTERIAL SECRETION

Post-translational control for secretion

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URLs

Pseudomonas aeruginosa
http://www.ncbi.nlm.nih.gov/sites/entrez?db=genomeprj&cmd=Retrieve&dopt=Overview&list_uids=12339

PAO1
http://www.ncbi.nlm.nih.gov/sites/entrez?Db=genomeprj&cmd=ShowDetailView&TermToSearch=331

Type VI secretion (T6S) in *Pseudomonas aeruginosa* undergoes post-translational regulation through threonine phosphorylation, according to a paper recently published in *Nature Cell Biology*.

P. aeruginosa strain PAO1 contains three T6S loci, known as Hcp secretion islands I–III (HSI-I–III). Researchers from the Mekalanos laboratory were interested in investigating the T6S system (T6SS) encoded by the HSI-I locus, which secretes Hcp1, and particularly in the functions of a serine–threonine kinase (PpkA) and a serine–threonine phosphatase (PppA) that are encoded by this locus.

They began by constructing *ppkA* and *pppA* deletion mutants in wild-type *P. aeruginosa* and in a Δ*rets* mutant strain. In the wild-type, the HSI-I system is expressed at low levels and there is no Hcp1 secretion, whereas in the Δ*rets* mutant the HSI-I system is overexpressed and there is hyper-secretion of Hcp1. In the wild-type background, the *ppkA* deletion had no effect on Hcp1, whereas the *pppA* deletion increased Hcp1 secretion. In the Δ*rets* background, deletion of *ppkA* abolished Hcp1 secretion and deletion of *pppA* increased Hcp1 secretion. Hcp1 was detected in periplasmic-enriched fractions from all of the mutants, suggesting that deletion of either *ppkA* or *pppA* blocks Hcp1 transport through the outer membrane.

The precise role of PpkA and PppA in T6S was probed using the AAA$^+$ ATPase ClpV1. It had previously been demonstrated that ClpVI is an essential component of the HSI-I T6S apparatus and that the distinct subcellular localization of ClpVI requires a functional T6SS. Using constructs in which ClpVI was fused to green fluorescent protein, the effect of the *ppkA* and *pppA* deletions on ClpVI localization was assessed. It was found that PpkA and PppA have reciprocal functions: in the absence of PpkA, the T6S foci were disrupted and there was a loss of Hcp1 secretion, whereas in the absence of PppA, the number of T6S foci increased and secretion was enhanced.

The discovery of these opposing activities led the authors to look for a common substrate for PpkA and PppA, and their attention was directed to forkhead-associated domain protein 1 (Fha1) as this protein is encoded by the T6S loci that also encode PpkA and PppA. *In vitro* and *in vivo* assays confirmed that Fha1 is a physiologically relevant substrate for both PpkA and PppA. How does Fha1 influence T6S? Localization studies showed that Fha1 co-localized to T6S foci, and the authors propose that Fha1 is a key scaffolding protein in the T6S apparatus.

The authors propose a model in which the secretion of Hcp1 by the HSI-I T6SS is subject to dynamic post-translational regulation. In the resting state, PpkA is inactive, and Fha1 is maintained in the dephosphorylated state by PppA. Once activated, PpkA phosphorylates Fha1, leading to the final assembly of the T6SS and secretion of Hcp1. More work remains to be done, including the identification of the signal that activates PpkA, but this exciting paper provides the first example of post-translational control of bacterial secretion.

Sheilagh Molloy

ORIGINAL RESEARCH PAPER Mougous, J. D. *et al.* Threonine phosphorylation post-translationally regulates protein secretion in *Pseudomonas aeruginosa*. *Nature Cell Biol.* **9**, 797–803 (2007)

FURTHER READING Mougous, J. D. *et al.* A virulence locus of *Pseudomonas aeruginosa* encodes a protein secretion apparatus. *Science* **312**, 1526–1530 (2007)