MshE: An Important Cyclic di-GMP Receptor and Its Involvement in Formation of a Biofilm of *Vibrio cholerae*

Mutating the highly conserved residues of MshE significantly diminish c-di-GMP binding and biofilm formation.

Cyclic dimeric GMP (c-di-GMP), a second messenger, is involved in vital cellular events, such as the formation of a biofilm, the biogenesis of pili and secretion of pathogenic factors in various bacteria.[1] Although the mechanisms of c-di-GMP biosynthesis and degradation have been studied thoroughly, the nature of c-di-GMP receptors and the process of c-di-GMP-mediated regulation remain elusive.

Recent work on MshE (from *Vibrio cholerae*) and its homologue PA14_29490 (from *Pseudomonas aeruginosa*) showed both to be potent c-di-GMP-binding ATPase. Both proteins utilize N- and C-terminal domains for c-di-GMP and ATP binding, respectively. Arg9 and Gln32 of MshE serve notably as c-di-GMP binding residues, but the two residues were not observed in any previously described canonical c-di-GMP binding motif. To discover whether MshE uses a special binding mode for c-di-GMP binding, a collaborative team led by Shan-Ho Chou (National Chung Hsing University) hence solved the complex structure of the N-terminal domain of MshE (*Vc*MshEN) with c-di-GMP using a 1.37-Å data set at **TLS 15A1** of NSRRC and **SP 44XU** of SPring-8.[2]

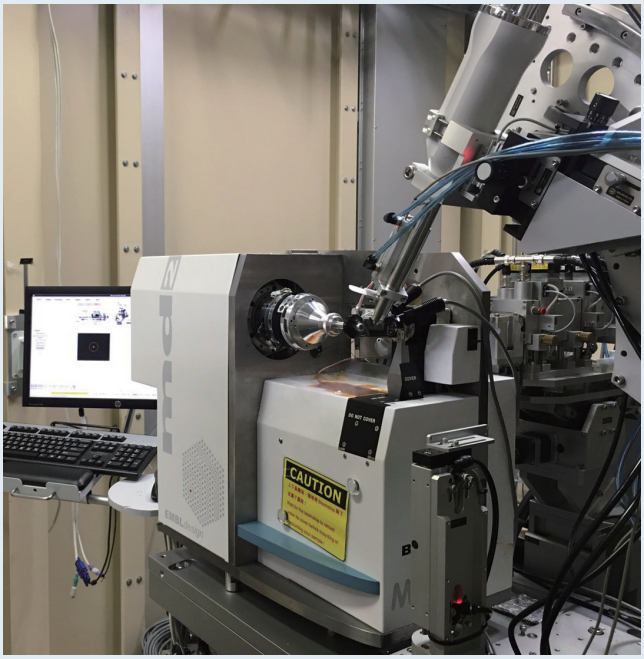

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Figure 1(a) shows that each MshEN structure comprises two subdomains, a four-helix domain (α1-α4, called MshEN_N) and an α/β domain (α5-α7 and β1-β3, called MshEN_C). To compare the two subdomains, the entire interaction between MshEN and c-di-GMP was contributed from MshEN_N except for Asp108 (**Fig. 1(b)**). c-di-GMP is thus bound mainly within the MshEN_N; in contrast, the role of MshEN_C in c-di-GMP binding requires further investigation. Next, to test whether the MshEN domain is specific for c-di-GMP binding, the authors altered the ligand from c-di-GMP to c-di-AMP. The big difference between them is that O^6 atom (H-bond acceptor) in c-di-GMP converts into a bulky amino group (H-bond donor), which causes a steric hindrance with the Asp12 amide proton of MshEN_N (**Fig. 1(c)**). Isothermal calorimetry (ITC) experiments proved that the MshEN domain cannot bind c-di-AMP because of the steric clashes (**Figs. 1(d) and 1(e)**). Differential scanning fluorimetry (DSF) was also performed to test whether other nucleotides containing adenine and guanine bases, including cAMP, ATP, ADP and AMP, and cGMP, GMP, GDP and GTP, are effective ligands for the MshEN domain. The DSF results confirmed clearly that the MshEN domain binds with no other nucleotide containing adenine or guanine.

Sequence alignment of the c-di-GMP-binding motifs showed that 12 residues, including R9, L10, G11, L25, L29, Q32, R38, L39, G40, L54, L58 and Q61, are highly conserved and play a key role in c-di-GMP binding. To investigate which conserved residues provided the greatest contribution to the c-di-GMP binding, the authors prepared a series of variants, such as single, double or triple MshEN_N mutants, for further testing using ITC and DSF. The ITC results revealed that hydrophobic interactions are important in c-di-GMP binding, because the mutated hydrophobic residues resulted in a binding affinity less than one tenth (**Figs. 1(f) and 1(g)**). To compare with preceding work, the mutated charged residues, including Arg9, Asp12 and Gln32, showed smaller effects on the c-di-GMP binding; the binding affinity seemed to decrease less than five-fold.[3] In contrast, the DSF results indicated that most hydrophobic residues with single, double

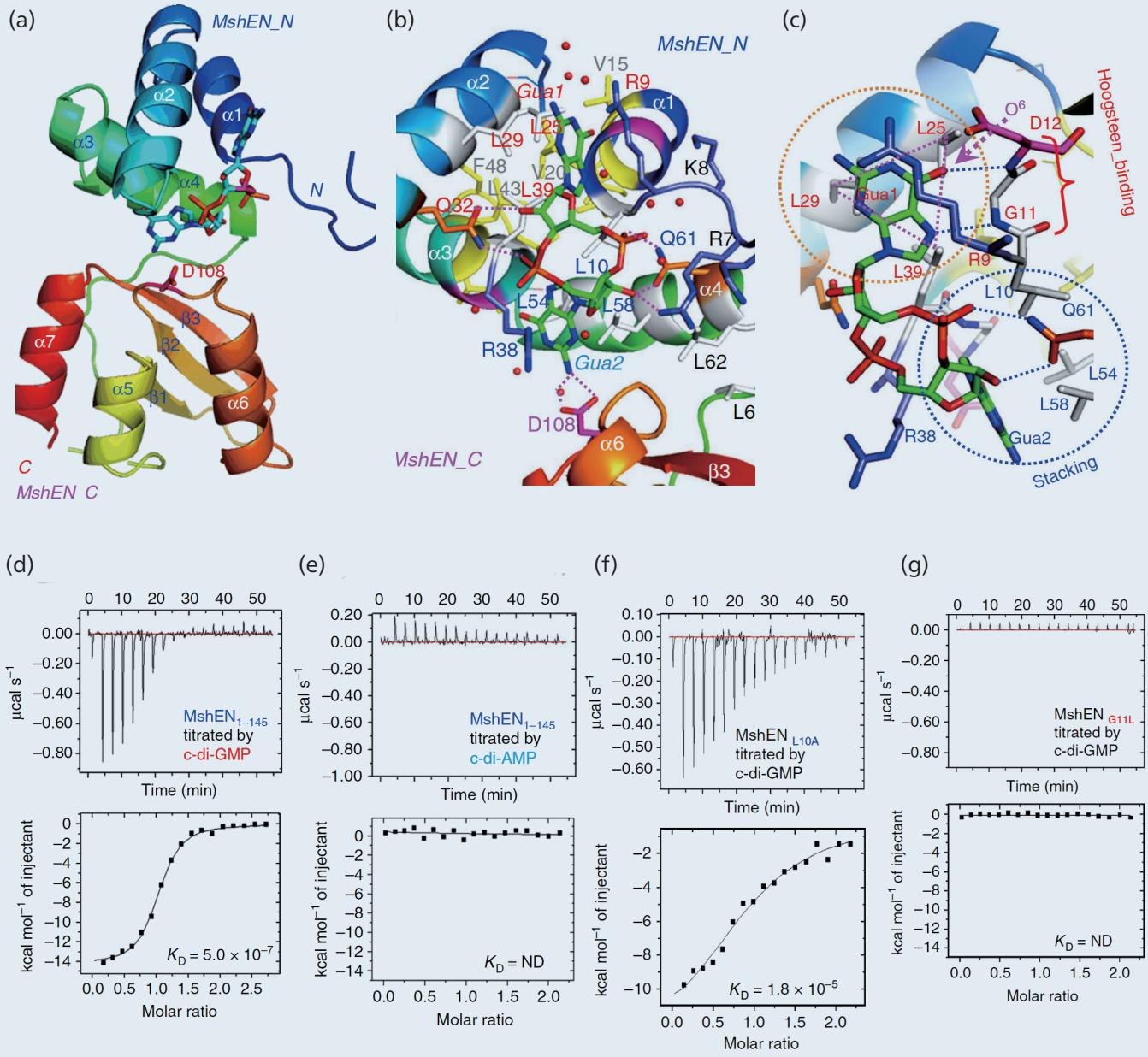

Fig. 1: Overall structure, binding mode and ITC assay of MshEN. (a) Two-subdomain structure, with c-di-GMP (shown in stick) bound primarily within the MshEN_N domain. (b) Interaction network of a MshEN-c-di-GMP complex. Water oxygen is shown as red sphere. (c) Hydrophobic interactions in c-di-GMP binding. Substitution of atom O^6 with an amino group of c-di-GMP would cause steric hindrance. (d) Tight binding between MshEN and c-di-GMP by ITC. (e) No detectable binding of c-di-GMP was observed with ITC. (f)-(g) L10A and G11L resulted in significantly decreased c-di-GMP-binding affinity with ITC. [Reproduced from Ref. 2]

and triple mutation were involved in the stability of this complex structure. In conclusion, the strong binding between MshEN and c-di-GMP results from a unique interaction mode.

The authors subsequently ascertained the biological importance of the hydrophobic residues participating in c-di-GMP binding through analysis of various phenotypes, including pilin production, biofilm formation and motility, in relation to the MshE function. Based on these results, the defective phenotypes resulting from lack of *mshE* were restored by the expression of wild-type *mshE in trans*. Some mutant-type *mshE*, including L10A, G11I and L10/58A, significantly affected, however, the ability of *ΔmshE* to perform normal phenotypes. (**Fig. 2**). Taken together, the results *in vitro* and *in vivo* indicated that binding of c-ci-GMP is crucial for MshE activity. (Reported by Chun-Hsiang Huang)

This report features the work of Shan-Ho Chou and his co-workers published in Nat. Commun. **7**, *12481 (2016)*

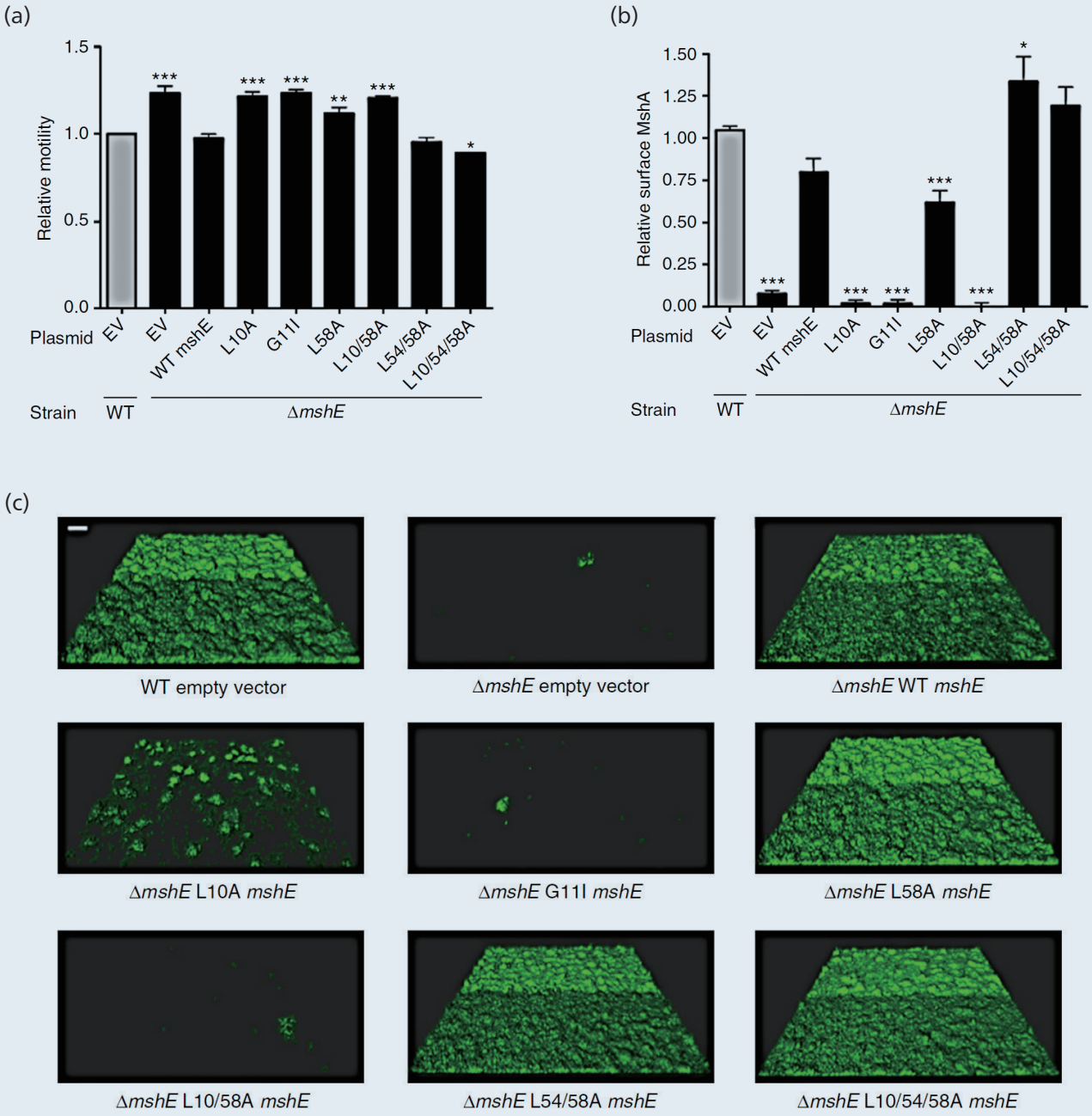

Fig. 2: Analysis of motility, pilus production and biofilm formation. *$P \leq 0.05$, **$P \leq 0.01$, *** $P \leq 0.001$. EV: empty vector. WT: wild-type. Δ*mshE*: *mshE* deletion mutant. [Reproduced from Ref. 2]

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- Protein Crystallography
- Biological Macromolecules, Protein Structures, Life Sciences

| References |

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