Conserved Structural Mechanisms for Autoinhibition in IpaH Ubiquitin Ligases*

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The IpaH family of novel E3 ligase (NEL) enzymes occur in a variety of pathogenic and commensal bacteria that interact with eukaryotic hosts. We demonstrate that the leucine-rich repeat (LRR) substrate recognition domains of different IpaH enzymes autoinhibit the enzymatic activity of the adjacent catalytic novel E3 ligase domain by two distinct but conserved structural mechanisms. Autoinhibition is required for the in vivo biological activity of two IpaH enzymes in a eukaryotic model system. Autoinhibition was retro-engineered into a constitutively active IpaH enzyme from Yersinia pestis by introduction of single site substitutions, thereby demonstrating the conservation of auto-regulatory infrastructure across the IpaH enzyme family.

The ubiquitin system regulates diverse cellular processes in all eukaryotes through the covalent attachment of ubiquitin to acceptor lysine residues on target substrates, thereby dictating protein stability, activity, and localization. Ubiquitin is activated and conjugated to its substrates by a sequential enzymatic cascade, E1 → E2 → E3 (1). E3 enzymes, also known as ubiquitin ligases, specifically recognize their substrates via protein interaction domains. The eukaryotic E3 enzymes fall into two major classes. The HECT* domain class facilitates transfer to substrates via a catalytic cysteine residue, whereas the RING/U-box domain class serves as a platform to bridge the E2 enzyme to the substrate.

The crucial role of the ubiquitin system in the regulation of host immune surveillance poises ubiquitin-dependent processes as a pervasive target for a wide range of pathogens (2). Several type III secretion virulence effectors in Gram-negative pathogenic bacteria possess ubiquitin ligase activity, including AvrPtoB from Pseudomonas syringae (3), SopA from Salmonella enterica typhimurium (4), NleG from enterohemorrhagic Escherichia coli (5), and the IpaH family members from Shigella flexneri and Salmonella enterica (6). AvrPtoB and NleG are structural mimics of RING/U-box domain ubiquitin ligases, whereas SopA is a structural mimic of HECT domain ubiquitin ligases (5, 7, 8). In contrast, IpaH enzymes possess a novel catalytic fold that does not resemble known eukaryotic E3 enzymes (9–11). The substrates for most pathogen-encoded E3 enzymes remain to be identified but are likely to function in host defense systems. For example, AvrPtoB and IpaH9.8, respectively, target the Fen and NF-κB essential modulator/IKB kinase γ (NEMO/IKKγ) protein kinases, both of which are key mediators of the innate immune response (12, 13).

The domain architecture of the IpaH family consists of a N-terminal leucine-rich repeat (LRR) domain and a C-terminal catalytic domain, termed the novel E3 ligase (NEL) domain (9). The NEL domain bears a cysteine residue that mediates transfer of ubiquitin to substrates through the formation of a thioester intermediate (10, 11), analogous to the structurally unrelated HECT domain in eukaryotes. The NEL domain is virtually identical (>98% identity) across all IpaH family members in Shigella and is highly conserved in other bacterial species. In contrast, the LRR domain that recognizes substrates is variable between IpaH family members and across bacterial species, which presumably reflects the diversity of specific target proteins. For instance, the LRR domain of the NEL enzyme SspH1 in Salmonella selectively targets PKN1 (14), whereas the LRR domain of Shigella IpaH9.8 targets NF-κB essential modulator/IκB kinase γ (13).

In addition to recruiting substrates, the LRR domain regulates the catalytic activity of the adjacent NEL domain. Removal of the LRR domain of IpaH9.8 or Salmonella SspH2 increases autoubiquitination and potentiates free polyubiquitin chain...
synthesis (9, 10, 11). The autoinhibitory effect of the LRR domain appears to be relieved through substrate interactions. For example, in the presence of the yeast surrogate substrate Ste7, autoubiquitination activity of the NEL domain of IpaH9.8 is enhanced (6). These observations suggest that substrate recognition is coupled to catalytic activation.

The mechanism by which the LRR domain regulates NEL domain catalytic activity has been suggested by recent structural studies (9, 10). To date, three crystal structures of NEL enzymes have been solved: the isolated NEL domain of Shigella IpaH1.4 (Protein Data Bank (PDB) number 3CKD) (10) and two near full-length structures encompassing the LRR and NEL domains of Shigella IpaH3 (S. flexneri strain 2a, PDB number 3CVR) (11) and Salmonella SspH2 (PDB number 3G06) (9). Notably, the higher order orientations adopted by the LRR and NEL domains of the IpaH3 and SspH2 enzymes are strikingly different. As the SspH2 structure corresponds to an autoinhibited state (9) and as autoinhibition was not detected for IpaH3 (11), the IpaH3 structure has been inferred to represent an active state (9). However, given that the close homologue IpaH9.8 (77% identity to IpaH3) does exhibit potent autoinhibition (10), we re-examined the autoinhibitory properties of a series of IpaH enzymes. We demonstrate that there are two distinct structural modes of NEL domain autoinhibition and that the inhibited versus non-inhibited states can be interconverted through facile substitution of key residues.

EXPERIMENTAL PROCEDURES

Constructs and Reagents—IpaH3 was PCR-amplified from S. flexneri strain 5a and cloned into pProEX. Y3400 was PCR-amplified from Yersinia pestis DNA and cloned into pProEX. IpaH9.8 and SspH1 were subcloned from pGEX constructs (6) into pProEX. The NEL domains of SspH1, IpaH9.8, and Y3400 consisted of residues 407–700, 254–545, and 303–605, respectively. Human UBE1 and ubiquitin were cloned into pETM-30. UBE2D3 was cloned into pProEX. PKN1 (IMAGE clone number 5752583) was cloned into a modified pET vector in fusion with an N-terminal His6-MBP (maltose-binding protein) tag. The destination vector pGAL1-cFLAG was used to create plasmids for expression of FLAG-tagged IpaH9.8 and SspH1 in yeast as described previously (10). Ubiquitin-K0 was purchased from Boston Biochem, anti-ubiquitin (P4D1) was from Covance, anti-PKN1 (N19) was from Santa Cruz Biotechnology, anti-His6 was from Qiagen, and anti-3-phosphoglycerate kinase (anti-PGK) was from Molecular Probes.

Protein Expression and Purification—Protein constructs were expressed in BL21 (DE3) CodonPlus and induced with 0.3 mM isopropyl-1-thio-β-D-galactopyranoside at 16 °C for overnight. Expressed proteins were purified using nickel-nitrilotriacetic acid affinity chromatography and stored in a buffer containing 20 mM HEPES (pH 7.5), 150 mM NaCl, 5% glycerol, and 1 mM DTT. Purification tags on UBE1, UBE2D3, and ubiquitin were removed by tobacco etch virus protease.

In Vitro Ubiquitination—Reactions were performed in a 20-μl reaction mixture containing 25 mM Tris-HCl (pH 7.5), 50 mM NaCl, 10 mM MgCl2, 5 mM ATP, 0.25 mM DTT, 1 μg of ubiquitin, 1 μM UBE1, 5 μM UBE2D3 (= UbcH5c), and 1 μM of indicated IpaH proteins in the presence or absence of 2 μM PKN1. Reactions were incubated at 25 °C for 60 min and stopped by SDS sample buffer containing 100 mM DTT. Reaction mixtures were separated by SDS-PAGE, transferred onto PVDF membrane, and probed with specific antibodies as indicated. Presented experiments were representative of minimally 3 independent replicates.

RESULTS

To investigate whether autoinhibition by the LRR domain is a general feature of NEL enzymes, we tested the previously uncharacterized Yersinia Y3400 enzyme for autoinhibition, along with selected Shigella and Salmonella enzymes. IpaH enzymes possess three specific ubiquitin transferase activities that can be monitored in vitro: (i) the polymerization of free ubiquitin chains; (ii) the ubiquitination of a substrate; and (iii) enzyme autoubiquitination. We first assayed the formation of free ubiquitin polymers as a measure of NEL domain enzymatic activity. As expected, deletion of the LRR domain liberated potent catalytic activity of the Shigella IpaH9.8 and Salmonella SspH1 NEL domains (Fig. 1A). Unlike a previous study (11), we also observed enhanced activation of the IpaH3 (S. flexneri strain 5a) catalytic domain. In contrast, we observed no effect of LRR domain deletion on the Yersinia Y3400 enzyme, which appeared constitutively active. Autoinhibition thus appears to be a feature of some but not all IpaH enzymes.

To understand the molecular basis for autoinhibition, we compared the three known IpaH structures. Consistent with the IpaH3 and SspH2 structures reflecting higher order off-states, their catalytic loop orientations differ markedly from that observed in the on-state structure of the isolated catalytic domain (Fig. 1B). However, the conformation of the catalytic loop also differed between the two off-state structures of full-length enzymes. To investigate whether these differences reflect diversity in autoinhibitory mechanisms across the IpaH family, we set out to design mutants to more precisely dissect the relevance of both the previously identified autoinhibitory mode revealed by the SspH2 structure (denoted mode 1) and...
FIGURE 1. Ubiquitin ligase activity and structures of selected IpaH enzymes. A, ubiquitination reactions with indicated full-length proteins or isolated NEL domains. Reaction products were detected with anti-ubiquitin antibody. B, structures of IpaH1.4 NEL (3CKD), IpaH3 (3CVR), and SspH2 (3G06) in ribbon representations. NEL domains are in different shades of blue, LRR domains are in red or purple, and the LRR-NEL linker is in orange. Below is a zoom-in of boxed catalytic loop regions with the catalytic cysteine circled in red. Dashed ribbons correspond to disordered regions. N indicates the N terminus. C and D, residues mediating the SspH2 (C) and IpaH3 (D) LRR-NEL interactions in the linker (orange), the LRR (purple or red), and the NEL (light or dark blue) domains. The catalytic loop (violet) and hydrogen bonds (yellow dots) are indicated. Corresponding residues of different IpaH homologues in SspH1 are shown in brackets (see supplemental Fig. S1 for alignments).
the second putative autoinhibitory mode revealed by the IpaH3 structure (denoted mode 2).

The interaction of the LRR and NEL domains in the SspH2 structure (mode 1) is bridged by the LRR-NEL linker; Ile-479, Phe-481, and Met-483 in the linker make hydrophobic contacts with Leu-465 in α4 of the LRR domain and with NEL domain residues Leu-638 in the αG-αH linker and Phe-667 and Val-670 in the αH-α1 linker (Fig. 1C). Additionally, Asp-482 in the linker hydrogen bonds with Ser-579 in the catalytic loop (αE-αF) of the NEL domain. In contrast, the LRR-NEL interaction in the IpaH3 structure (mode 2) is mediated by direct contacts between the two domains. Notably, Phe-421 in the αG-αH linker of the NEL domain interacts with Ile-222 at the center of a hydrophobic pocket on the LRR domain (Fig. 1D). Hydrogen bonds are also observed between main-chain amino groups of Phe-421 and His-420 in the αG-αH linker with Glu-224 in α3, between Ser-454 in the αG-αH linker with His-221 in the αH-α1 linker, and between Asp-321 in αC with Ser-244 in α4, within the NEL and LRR domains, respectively. Although the mode 1 versus mode 2 structures appear quite dissimilar, a common feature is the involvement of the NEL domain residue at the apex of the αG-αH linker (Leu-638 in SspH2 and Phe-421 in IpaH3). With this in mind, we designed a set of mutants to selectively test the functional relevance and generality of the two intramolecular binding modes for autoinhibition.

We generated mutations in two representative IpaH homologues, Shigella IpaH9.8 and Salmonella SspH1, and tested for effects on polyubiquitin chain synthesis in vitro. Consistent with previous findings for Salmonella SspH2 (9), an I391D substitution (corresponding to Ile-479 in SspH2) or the double F393A/M395A substitution (corresponding to Phe-481/Met-483 in SspH2) circumvented the mode 1 autoinhibition in SspH1 (Fig. 2A). Introduction of the analogous substitutions in the more divergent Shigella IpaH9.8 enzyme also abrogated autoinhibition. Thus, the autoinhibitory mechanism represented in the SspH2 structure (mode 1) appears to operate in other IpaH family enzymes.

We next probed the predicted autoinhibitory mode 2 contacts in the two representative IpaH homologues, IpaH9.8 and SspH1. Single site substitutions of either of the domain contact residues, F395R within the NEL domain and the reciprocal substitution I196D in the LRR domain of Shigella IpaH9.8, effec-
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reactively abrogated autoinhibition (Fig. 2B). This result suggested that the mode 2 conformation also reflects an autoinhibited state, rather than an active state as inferred previously (11). Surprisingly, introduction of the analogous mode 2 mutations in Salmonella SspH1 also relieved autoinhibition. The Phe residue at the apex of the αG-αH linker of the IpaH3 structure is occupied by a conserved Leu residue in SspH1. A conservative L550F mutation had no effect on autoinhibition of SspH1, whereas a non-conservative L550S mutation overcame autoinhibition (Fig. 2B). Thus, autoinhibitory mode 2 revealed by the IpaH3 structure also applies to other IpaH enzymes.

To verify that mutations disrupting LRR-NEL interfaces function through relieving the LRR-mediated autoinhibition, the L550S and catalytically inactive (C492A) mutants. Right panel, polyubiquitin chain formation by isolated WT IpaH9.8 NEL domain and F550R and catalytically inactive (C337A) mutants. B, autoubiquitination of WT and indicated interface mutant of SspH1 (left panel) and IpaH9.8 (right panel) using a chain terminating variant of ubiquitin (Ub-K0). C, polyubiquitination of PKN1 by the indicated SspH1 mutants.

FIGURE 3. Autoinhibition of IpaH enzyme activity prevents polyubiquitin chain formation and autoubiquitination. A, left panel, polyubiquitin chain formation by isolated wild type (WT) SspH1 NEL domain and L550S and catalytically inactive (C492A) mutants. Right panel, polyubiquitin chain formation by isolated WT IpaH9.8 NEL domain and F550R and catalytically inactive (C337A) mutants. B, autoubiquitination of WT and indicated interface mutant of SspH1 (left panel) and IpaH9.8 (right panel) using a chain terminating variant of ubiquitin (Ub-K0). C, polyubiquitination of PKN1 by the indicated SspH1 mutants.

Coupling of substrate recognition to catalytic activation of E3 ubiquitin ligases is thought to prevent promiscuous ubiquitination of unintended substrates and/or enzyme degrada-

tion due to autoubiquitination (15). To determine whether this regulatory principle operates in IpaH enzymes, we examined SspH1 and IpaH9.8 interface mutants in autoubiquitination assays. Mutations that activated polyubiquitin chain synthesis also promoted autoubiquitination of both SspH1 and IpaH9.8 enzymes (Fig. 3B). Disruption of the LRR-NEL interface did not affect the ability of SspH1 to ubiquitinate its endogenous human substrate PKN1 (Fig. 3C). Therefore, autoinhibition of IpaH enzymes may serve in part to restrict catalytic activity toward specific physiological targets while preventing ubiquitination of the enzymes themselves.

We previously established a FUS1pr-HIS3 strain of the budding yeast Saccharomyces cerevisiae as a surrogate biological model for IpaH9.8 enzyme function (6). To examine the relevance of autoinhibition for IpaH function in vivo, we conditionally expressed IpaH9.8 from the GAL1 promoter in this established FUS1pr-HIS3 yeast strain (Fig. 4A). As reported previously (6), IpaH9.8 inhibited the mating pheromone pathway by eliminating the Ste7 MAPK kinase, thereby preventing expression of a FUS1pr-HIS3 reporter construct and causing the consequent inability to grow on −His medium. Interestingly, SspH1 caused a severe growth defect in a wild type yeast strain.
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FIGURE 4. Biological consequences of autoinhibition. A, serial dilution growth analysis of a FUS1pr-HIS3 yeast reporter strain expressing wild type (WT) GAL1pr-IpaH9.8FLAG or catalytically inactive mutant (C337A) or LRR-NEL interface mutants (F395R, I196D, I238D). 

B, serial dilution growth analysis of a wild type yeast strain expressing wild type (WT) GAL1pr-SspH1FLAG construct or catalytically inactive (C492A) mutant or LRR-NEL interface mutants (L550S, L352D, I391D). Lower panel, expression levels of IpaH9.8 proteins were confirmed by immunoblot. PGK, 3-phosphoglycerate kinase. B, serial dilution growth analysis of a wild type yeast strain expressing wild type (WT) GAL1pr-SspH1FLAG construct or catalytically inactive (C492A) mutant or LRR-NEL interface mutants (L550S, L352D, I391D). Lower panel, expression levels of SspH1 proteins were confirmed by immunoblot. WT, C492A, L550S, L352D, I391D, GAL1pr-IpaH9.8FLAG, GAL1pr-SspH1FLAG. 

C, protein stability analysis of indicated constructs were grown in raffinose (Raf)-containing medium, induced by galactose for 1 h, and then resuspended in medium containing glucose (Glu) and cycloheximide (CHX). SspH1FLAG and IpaH9.8FLAG protein levels were detected by anti-FLAG antibody and normalized against a housekeeping protein (PGK) at the indicated time points upon treatment with glucose and cycloheximide.

We then addressed the mechanism by which NEL enzymatic activity is perturbed by autoinhibition. The binding of IpaH9.8 to ubiquitin-charged E2 enzyme facilitates discharge of the ubiquitin thioester in a manner that depends on the NEL domain active site cysteine (6). Full-length SspH1 and the constitutively active isolated NEL domain, or isolated catalytically inactive NEL domain (NELC492A), isolated NEL domain, or isolated catalytically inactive NEL domain (NELC492A), catalyze ubiquitination process, highlighting possible steps at which autoinhibition is achieved. 1) NEL domain engages charged E2; 2) NEL domain accepts transfer of Ub from the E2 to the active site cysteine on the E3; 3) NEL domain catalyzes transfer of Ub from its active site cysteine to an acceptor lysine in the substrate with substrate corresponding to an exogenous protein engaged by the LRR domain in the case of full-length IpaH enzyme or to ubiquitin in the case of the isolated NEL domain and to an exogenous protein, ubiquitin, and the IpaH enzyme itself in the case of constitutively active IpaH mutants. N indicates the N terminus.

such as the Y3400 enzyme of Y. pestis. In Y3400, the residue corresponding to the critical SspH1 Leu-550 is polar in nature and analogous to the activating L550S substitution in SspH1 (supplemental Fig. S1), thereby explaining the absence of autoinhibition in the Yersinia enzyme. However, Y3400 does possess a hydrophobic pocket in the LRR domain and the bridging Ile residue in the LRR-NEL linker, both of which are compo-

background (Fig. 4B), presumably by targeting one or more unidentified essential proteins for degradation. Mutations that disrupt either of the modes of autoinhibition in vitro abrogated toxicity of both IpaH enzymes in the respective surrogate genetic assays. Consistent with the possibility that autoinhibition prevents unintended autoubiquitination and destruction of the IpaH enzyme itself, the in vivo half-lives of constitutively active IpaH enzyme mutants were somewhat decreased (Fig. 4C and supplemental Fig. S3). Autoinhibition by either mode 1 or mode 2 can therefore regulate IpaH enzyme function in vivo.

If the autoregulatory features of the IpaH9.8 and SspH1 enzymes demonstrated above are generally applicable, it should be possible to explain the regulatory features of other IpaH enzymes in structural terms and to engineer artificial autoinhibitory mechanisms into enzymes that lack autoinhibition,
nents of the autoinhibitory mechanism in other enzymes. We retro-engineered an artificial autoinhibitory interaction between the LRR and NEL domains of Y3400 by introducing a S449F mutation in the apex of the αG-αH linker. This mutation did not adversely affect the catalytic activity of the isolated Y3400 NEL domain but substantially reduced activity of the full-length Y3400 enzyme (Fig. 6A). Importantly, the engineered autoinhibition of Y3400S449F was abolished by mutations that disrupt either of the two modes of autoinhibitory interactions in SspH2 and IpaH3 (Fig. 6, B and C). These results demonstrate that the LRR-NEL domain architecture of Y3400 retains the capacity for autoinhibition through mode 2 hydrophobic pocket interactions, as in IpaH3, and/or through mode 1 bridging linker interactions, as in SspH2.

**DISCUSSION**

Eukaryotic ubiquitin ligases are regulated through diverse mechanisms. For example, the catalytic efficiency of the cullinRING ligase enzymes is activated by conjugation of the ubiquitin-like modifier Nedd8, interactions with inhibitory factors, and dimerization (16). HECT domain E3 enzymes are similarly subject to multiple levels of control, including autoinhibition through higher order intramolecular interactions of the HECT domain with flanking interaction domains (17). For a number
of E3 enzymes, autoubiquitination mediates proteasome-dependent degradation of the E3 itself, which intrinsically self-limits enzymatic activity (15). The overall weak effect on protein stability by disrupting LRR-TEL interaction in yeast models (supplemental Fig. S3) suggests that autoregulation may influence biological functions of IpaH enzymes through additional mechanisms.

Our results demonstrate that the two outwardly dissimilar higher order structures displayed by the IpaH3 and SspH2 enzymes reflect two different modes of autoinhibition. These modes differ in the relative positioning of the LRR domain with respect to the NEL domain. In mode 1, the concave surface of the LRR domain orients toward the NEL domain, whereas in mode 2, the concave surface is oriented opposite to the NEL domain. Despite this overt structural difference, in both modes, the LRR-TEL interaction impinges on the active site region of the NEL domain to down-regulate catalytic function. Based on our mutational analysis, both inhibitory modes appear to operate in the same enzyme, suggesting that the two autoinhibited states exist in equilibrium. The observation that mutations that disrupt mode 1 interactions are more potent activators than mutations that disrupt mode 2 interactions (supplemental Fig. S2) suggests that mode 1 might be the predominant conformation that is sampled in solution. These distinct autoregulatory orientations may also allow plasticity in how the LRR domain engages substrates and/or how substrate interactions alleviate autoinhibition. Future structural work of reaction intermediates will be required to understand precisely how the LRR domain engages substrates and/or how substrate interactions alleviate autoinhibition. Future structural work of reaction intermediates will be required to understand precisely how the LRR domain inhibits the cata
tic transfer of ubiquitin from the NEL domain to substrates and how the autoinhibition of IpaH enzymes influences pathogen virulence.

Autoinhibition is a conserved structural and regulatory feature of IpaH enzymes, although it is latent in some enzymes, such as Yersinia Y3400. As noted above, autoinhibition may prevent the ubiquitination of unintended protein substrates and/or preclude autoubiquitination that might otherwise lead to destruction or inactivation of the enzyme itself. Recent studies suggest another advantage of autoinhibition, namely to allow pathogenic bacteria to escape the host surveillance system that detects untethered ubiquitin chains (18). These products of pathogen-encoded E3 enzymes can activate the TAK1 and IκB kinase (IKK) kinase complexes, which in turn activate NF-κB and the innate immune response (18). The elucidation of full-length LRR-TEL enzyme structures bound to different substrates should reveal how substrate engagement alleviates catalytic constraints of the autoinhibited structure. In turn, these interdomain interactions might be targeted to mitigate pathogen virulence.

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REFERENCES
1. Pickart, C. M. (2001) Annu. Rev. Biochem. 70, 503–533
2. Hicks, S. W., and Galán, J. E. (2010) Curr. Opin. Microbiol. 13, 41–46
3. Abramovitch, R. B., Janjusevic, R., Stebbins, C. E., and Martin, G. B. (2006) Proc. Natl. Acad. Sci. U.S.A. 103, 2851–2856
4. Zhang, Y., Higashide, W. M., McCormick, B. A., Chen, J., and Zhou, D. (2006) Mol. Microbiol. 62, 786–793
5. Wu, B., Skarina, T., Yee, A., Jobin, M. C., Dileo, R., Semesi, A., Fares, C., Lemak, A., Coombes, B. K., Arrowsmith, C. H., Singer, A. U., and Savchenko, A. (2010) PLoS Pathog. 6, e1000960
6. Rohde, J. R., Breitkreutz, A., Chenal, A., Sansonetti, P. J., and Parson, C. (2007) Cell Host Microbe 1, 77–83
7. Diao, J., Zhang, Y., Hübregtsse, J. M., Zhou, D., and Chen, J. (2008) Nat. Struct. Mol. Biol. 15, 65–70
8. Xing, W., Zou, Y., Liu, Q., Liu, J., Luo, X., Huang, Q., Chen, S., Zhu, L., Bi, R., Hao, Q., Wu, J. W., Zhou, J. M., and Chai, J. (2007) Nature 449, 243–247
9. Quezada, C. M., Hicks, S. W., Galán, J. E., and Stebbins, C. E. (2009) Proc. Natl. Acad. Sci. U.S.A. 106, 4864–4869
10. Singer, A. U., Rohde, J. R., Lam, R., Skarina, T., Kagan, O., Dileo, R., Chirgadze, N. Y., Cuff, M. E., Joachimiak, A., Tyers, M., Sansonetti, P. J., Parson, C., and Savchenko, A. (2008) Nat. Struct. Mol. Biol. 15, 1293–1301
11. Zhu, Y., Li, H., Hu, L., Wang, J., Zhou, Y., Pang, Z., Liu, L., and Shao, F. (2008) Nat. Struct. Mol. Biol. 15, 1302–1308
12. Ntoukakis, V., Mucyn, T. S., Gimenez-Ibanez, S., Chapman, H. C., Gutierrez, I. R., Balmuth, A. L., Jones, A. M., and Rathjen, J. P. (2009) Science 324, 784–787
13. Ashida, H., Kim, M., Schmidt-Supprian, M., Ma, A., Ogawa, M., and Sasaki, C. (2010) Nat. Cell Biol. 12, 66–73
14. Haraga, A., and Miller, S. I. (2006) Cell Microbiol. 8, 837–846
15. Petrovski, M. D., and Deshaies, R. J. (2008) Nat. Rev. Mol. Cell Biol. 6, 9–20
16. Merlet, J., Burger, J., Gomes, J. E., and Pintard, L. (2009) Cell Mol. Life Sci. 66, 1924–1938
17. Wiesner, S., Ogunjimi, A. A., Wang, H. R., Rotin, D., Sicheri, F., Wrana, J. L., and Forman-Kay, J. D. (2007) Cell 130, 651–662
18. Xia, Z. P., Sun, L., Chen, X., Pineda, G., Jiang, X., Adhikari, A., Zeng, W., and Chen, Z. J. (2009) Nature 461, 114–119