STRUCTURAL BIOLOGY

Enzyme illuminates bacterial ubiquitination

Structural analysis reveals how a bacterial enzyme catalyses attachment of the protein tag ubiquitin to host proteins, illuminating a process that allows pathogenic bacteria to subvert host–cell function. SEE ARTICLE P.674 & LETTERS P.729 & P.734

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Ubiquitination is a type of protein modification in which the protein ubiquitin is attached to a target protein. In eukaryotes (organisms that include fungi, plants and animals), the addition of a ubiquitin tag can act as a signal for various cellular processes. A prime example is the destruction of ubiquitinated proteins by a eukaryotic protein complex called the proteasome. The ubiquitination process is also the target of many bacterial pathogens, which have developed techniques to hijack it for their own benefit. In papers in *Nature*, Akturk *et al.*[1] (page 729), Dong *et al.*[2] (page 674) and Kalayil *et al.*[3] (page 734) describe the X-ray crystal structure of the bacterial enzyme SdeA, which catalyses ubiquitination. And, writing in *Cell*, Wang *et al.*[4] report the structure of a bacterial enzyme called SidE from the same protein family as SdeA.

The eukaryotic ubiquitination pathway requires a three-enzyme cascade[5]. An enzyme called E1 activates ubiquitin using a molecule of ATP and a magnesium ion (Mg^{2+}) to covalently bind the ubiquitin through a type of linkage called a thioester bond. The activated ubiquitin is then transferred to downstream enzymes, which attach ubiquitin through an isopeptide bond to a lysine amino-acid residue in the target protein. The discovery[6] of the SidE family of ubiquitin ligase enzymes in the bacterial pathogen *Legionella pneumophila* revealed a ubiquitination pathway with striking differences from the eukaryotic system. Not only can SidE ligases carry out the complete process without the aid of other enzymes, but this pathway also generates a different form of ubiquitin, termed phosphoribosylated ubiquitin (PR-Ub), in which a phosphoribose-sugar linkage attaches ubiquitin to the target protein[7].

The bacterial ubiquitination pathway requires a molecule of NAD^+ instead of the ATP and Mg^{2+} used by eukaryotes. In the first step, the mono-ADP-ribosyltransferase (mART) domain of SdeA uses ubiquitin and NAD^+ to covalently attach an adenosine diphosphate ribose (ADPR) molecule to an arginine residue (Arg42) of ubiquitin[6], producing an ADPR-Ub molecule. The phosphodiesterase (PDE) domain of SdeA then cleaves this ADPR-Ub to release the molecule AMP, generating PR-Ub, and this group forms a bond with a serine residue in the target protein[7]. However, the molecular details of how ubiquitination is catalysed were a mystery until now.

The four papers[1–4] provide a detailed picture of the bacterial reaction pathway, with complementary insights into the catalytic mechanisms. Akturk, Dong and Kalayil, and their respective colleagues, report atomic structures of SdeA's catalytic core, which consists of the PDE and mART domains.

Dong *et al.* imaged the largest fragment of SdeA, which includes part of the protein's carboxy-terminal domain. They observed that the C-terminal domain is required to anchor the PDE and mART domains, stabilizing the enzyme in an active conformation. The structure of SidE reported by Wang and colleagues reveals that its catalytic domains are similar to those of SdeA, but the authors conclude that the C-terminal domain mediates SidE dimerization. This disparity might result from differences in the experimental conditions used by the two groups, or might reflect specialized functions of the individual SidE family members.

The generation of ADPR-Ub from ubiquitin and NAD^+ in the first step of the reaction is revealed in a structure presented by Dong *et al.* of the mART domain in complex with ubiquitin and the molecule NADH, which is similar to NAD^+ but can inhibit catalysis by the enzyme. This revealed that the Arg42 residue in ubiquitin that becomes modified is located too far away from the ribose group of NADH for modification to occur directly. By contrast, another of ubiquitin's arginine residues, Arg72, which was previously shown to be important in SdeA-mediated ubiquitination[7], is located much closer to the enzyme-bound NADH. The authors used computer simulations of the complex, called molecular dynamics, to show that Arg72 and one other arginine residue (Arg74) anchor ubiquitin to mART. Once the nicotinamide group from NADH is released from the enzyme, a conformational change can occur, allowing Arg42 to replace Arg72 in the active site. This model explains why ADPR attaches selectively to Arg42 and not to

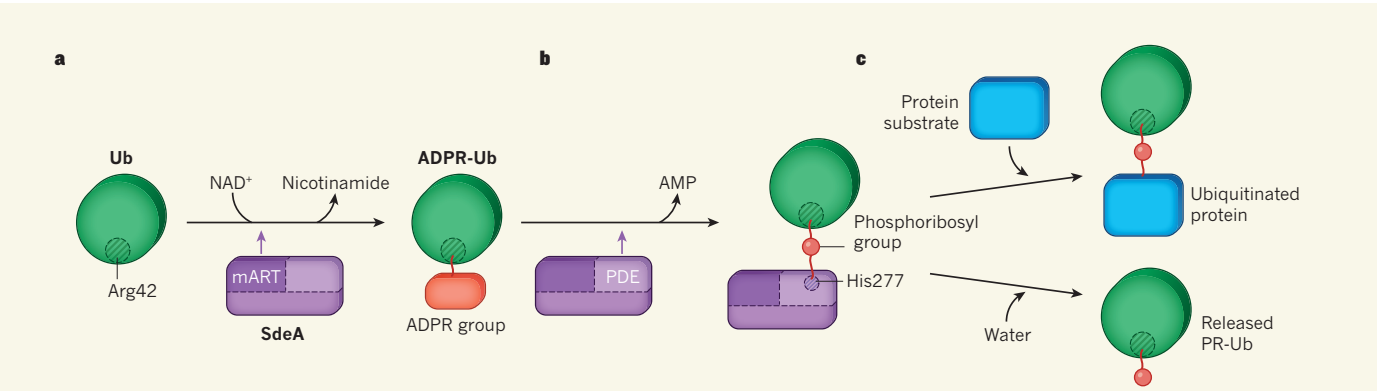

Figure 1 | The ubiquitination mechanism used by bacteria. Four papers[1–4] provide complementary insights into how bacterial enzymes from the SidE family mediate the process in which the protein ubiquitin (Ub) is attached to a target protein. Akturk *et al.*[1], Dong *et al.*[2] and Kalayil *et al.*[3] report the structure of the enzyme SdeA, and Wang *et al.*[4] present the structure of the enzyme SidE. **a**, In the first step, the enzyme's mART domain processes NAD^+ and adds an adenosine diphosphate ribose (ADPR) group to the amino-acid residue arginine 42 (Arg42) of ubiquitin. This generates ADPR-Ub in a reaction that releases nicotinamide. Dong *et al.*[2] reveal that Arg72 of ubiquitin (not shown) helps to anchor ubiquitin to the enzyme. **b**, ADPR-Ub is then processed by the enzyme's PDE domain. The molecule AMP is released in a reaction that generates ubiquitin bound to a phosphoribosyl group (PR-Ub); the phosphoribosyl group, in turn, is covalently attached to the enzyme's amino-acid residue histidine 277 (His277). **c**, If a protein substrate enters the enzyme's active site, the enzyme catalyses the attachment of PR-Ub to a serine residue (not shown) on the protein substrate. If, instead, water enters the active site, PR-Ub is released.

other arginines in ubiquitin, but further study is warranted to fully understand the process.

As the reaction proceeds, ADPR-Ub is processed by the PDE domain and PR-Ub is attached to a serine residue on a substrate protein. In addition to their studies of SdeA, Akturk *et al.* present the structure of ADPR-Ub in complex with SdeD, a member of the SidE family that contains only a PDE domain. Kalayil *et al.* used mass spectrometry techniques to study the SdeA catalytic intermediates at this stage. Both groups propose a two-step reaction mechanism for SdeA on the basis of studies of SdeA or SdeD.

First, the Glu340 amino-acid residue of SdeA binds ADPR-Ub. The His277 residue of SdeA interacts with a phosphate group on ADPR-Ub, resulting in the release of a molecule of AMP. Second, His407 activates the hydroxyl group of a serine residue on the target protein, which enables the attachment of PR-Ub to the serine. Using a mutated version of SdeA in which the histidine residue at position 407 was replaced with asparagine to trap a catalytic intermediate, Kalayil *et al.* captured PR-Ub bound to His277 of SdeA (Fig. 1), confirming the catalytic mechanism. Wang *et al.* report the structures of related complexes of ADPR and ubiquitin with SidE.

If a water molecule enters the PDE domain's active site instead of a serine amino-acid residue, the reaction product released is unbound PR-Ub. PR-Ub can inhibit host E1-dependent ubiquitination because the PR modification prevents this form of ubiquitin from being a substrate for eukaryotic ubiquitination enzymes[7]. Kalayil *et al.* answered the question of whether the pathogenicity associated with SdeA arises from the generation of unbound PR-Ub or from the ubiquitination of host proteins. The authors tested bacterial mutants lacking SidE proteins that were engineered to express either wild-type SdeA or a mutant version of SdeA that generates only unbound PR-Ub. The authors observed that the bacteria that express mutant SdeA were unable to grow in host cells, indicating that the enzyme's key role is ubiquitination of host proteins.

The role of PR-Ub is an emerging topic in the field of ubiquitin research. These structures of SidE family members now pave the way for more questions to be answered. For example, how is ADPR-Ub shuffled between the PDE and mART domains? The active sites of the PDE and mART domains are far apart (55 ångströms) and do not face each other. There is conflicting evidence as to whether SidE proteins exist as monomers or dimers, and, as a result, there are different models of how the gap between the domains might be bridged.

And what range of functions does the enzyme's C-terminal domain have? The C-terminal domain stabilizes the catalytic core in SdeA but mediates protein dimerization in SidE. Dong *et al.* observed that ubiquitin molecules bind to the C-terminal domain of SdeA and induce a large conformational change in the enzyme, which suggests a possible regulatory role for this domain.

How many host proteins are ubiquitinated by SidE-family ligases? So far, only a few SdeA substrates have been identified[6,8,9]; these include the GTPase enzymes Rab and Rag, as well as the protein RTN4. From analysis of the ubiquitination sites in host proteins, Kalayil *et al.* and Wang *et al.* propose that the ligase enzyme specifically targets serine residues in disordered protein regions.

Finally, perhaps the most exciting question still to be answered is this: do enzymes that mediate this type of ubiquitination process also exist in eukaryotes? ■

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QUANTUM MATERIALS

Spinning on the edge of graphene

Long-sought evidence has been found of magnetism at the edges of graphene, a two-dimensional form of carbon. The findings might enable the development of the logic gates needed for quantum computers. SEE LETTER P.691

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The 2D form of carbon known as graphene has many potentially useful properties, but is usually not magnetic when pristine. However, theoretical predictions suggest that the edges of graphene sheets should become magnetic when they have a zigzag arrangement of carbon atoms[1]. Observing this effect has been challenging because of the difficulties of detecting the predicted minute magnetic signal and because it is hard to fabricate defect-free edges that have the required shape. On page 691, Slota *et al.*[2] report a method for making nanometre-wide graphene ribbons in solution, and thereby for producing nanoribbons with well-defined zigzag edges 'decorated' with organic radical molecules that bear electron spins — a quantum property of electrons that is associated with magnetism. The authors' results provide solid evidence of magnetism at graphene edges, and show that edge spins have potentially useful quantum dynamics.

Magnetic forms of graphene would be useful for spintronics, a technology that forms the basis of today's magnetic data storage[3,4]. But the main interest in generating magnetic edge states in graphene is for quantum technologies. Electron spins can adopt two orientations relative to an external magnetic field, and these could be used to encode the '0' and '1' states of a quantum bit (qubit), the basic information unit of future quantum computers and quantum-simulation devices.

The quantum states of a qubit must be strongly coupled to external control stimuli that drive the qubit's operation, but they must also be isolated from random external perturbations that can irreversibly upset the 'coherent' evolution of such quantum states (coherence is the existence of non-classical correlations between quantum states). In these respects, graphene has potential advantages[5] over other materials that are being investigated as hosts for spin qubits, such as gallium arsenide or silicon: electric currents flowing through a graphene sheet provide a means of coupling and manipulating spins; and the two main sources of decoherence are minimal in graphene. These sources of decoherence are the coupling between an electron's spin and its orbital motion (which is weak in graphene), and interactions of electron spins with atoms that have nuclear spins (the concentration of which is low in graphene).

Why has it been so difficult to observe magnetic edge states experimentally? The electronic and magnetic properties of graphene nanoribbons correlate closely with the structures of their edges, and are sensitive to even minute numbers of defects. Isolating a sufficient number of nanoribbons that have perfect zigzag edges to enable their magnetic characterization is extremely challenging, and so the data from such studies[6] are scarce and inconclusive. Experiments performed on single graphene layers prepared *in situ* under a high vacuum have revealed the formation of local electronic states at edges, but did not provide any evidence of magnetism[7].

By expanding a previously developed