either non-doped or conventionally doped spiro-OMeTAD. More importantly, CO_2 bubbling decreases the doping time to just one minute. Having such a short doping time will be essential for the commercial production of perovskite solar cells.

Because the lithium carbonate by-product is removed by filtration, the density of lithium ions in the resulting HTL is lower than that in HTLs made using conventional doping. Kong *et al.* show that solar cells prepared using their method are much more stable than are control devices prepared using conventional doping, consistent with the smaller amount of lithium in the HTL. The authors' solar cells retain about 80% of their initial power-conversion efficiency after 500 hours of continuous operation, whereas the efficiency of the control devices rapidly drops to less than 75% after just 6 hours.

Even more excitingly, Kong and colleagues report that their CO_2-bubbling method is not limited to small-molecule organic semiconductors such as spiro-OMeTAD — the conductivity of a broad range of polymeric organic semiconductors (mixed with LiTFSI) is between 2 and 100 times greater than that of polymer–LiTFSI films not treated with CO_2. The power-conversion efficiency of perovskite solar cells made using the resulting doped polymeric HTLs is also substantially increased.

Perovskite solar cells show great promise, and have the potential to find industrial applications in the next few years. However, mass-manufacturing methods must first be developed that produce devices with long-term stability. This means that any compounds that reduce performance must be rigorously removed from HTLs during manufacturing. Kong and colleagues' work is important because it shows the feasibility of removing stability-lowering compounds using potentially scalable doping methods. So, although the power-conversion efficiency and stability of the authors' devices are not the best in the field, the new findings will inspire the development of other advanced doping strategies for rapidly producing clean films of organic semiconductors — thereby accelerating the pace of commercialization of perovskite solar cells.

Jianfeng Lu is at State Key Laboratory of Silicate Materials for Architectures, Wuhan University of Technology, 430070 Wuhan, China, and at the Foshan Xianhu Laboratory of the Advanced Energy Science and Technology Guangdong Laboratory, Foshan, China. **Fuzhi Huang** is at State Key Laboratory of Advanced Technology for Materials Synthesis and Processing, Wuhan University of Technology, Wuhan 430070, China, and at the Foshan Xianhu Laboratory of the Advanced Energy Science and Technology Guangdong Laboratory, Foshan, China.
e-mail: jianfeng.lu@whut.edu.cn

1. Jeong, J. *et al. Nature* **592**, 381–385 (2021).
2. Schloemer, T. H., Christians, J. A., Luther, J. M. & Sellinger, A. *Chem. Sci.* **10**, 1904–1935 (2019).
3. Kong, J. *et al. Nature* **594**, 51–56 (2021).
4. Salzmann, I., Heimel, G., Oehzelt, M., Winkler, S. & Koch, N. *Acc. Chem. Res.* **49**, 370–378 (2016).
5. Forward, R. L. *et al. ACS Energy Lett.* **4**, 2547–2551 (2019).
6. Ren, G. *et al. J. Mater. Chem. A* **9**, 4589–4625 (2021).
7. Tan, B. *et al. Adv. Energy Mater.* **9**, 1901519 (2019).
8. Li, Z. *et al. Energy Environ. Sci.* **10**, 1234–1242 (2017).
9. Hu, M. *et al. ACS Appl. Mater. Interfaces* **12**, 8260–8270 (2020).
10. Yin, X., Song, Z., Li, Z. & Tang, W. *Energy Environ. Sci.* **13**, 4057–4086 (2020).

The authors declare no competing interests.

Cell biology

Host ubiquitin protein tags lipid to fight bacteria

Brenda A. Schulman & J. Wade Harper

Host cells battle invading bacteria using a degradation process facilitated by the protein ubiquitin. The discovery of the host enzyme responsible and its bacterial target reveals that this process defies convention. **See p.111**

Intracellular bacteria such as *Salmonella* are a major threat to human health. These disease-causing microorganisms enter human cells cloaked in a host-derived membrane, which forms a structure termed a vacuole. To proliferate, *Salmonella* then need to access the cytoplasm, a feat that they typically manage through vacuole rupture. On page 111, Otten *et al.*[1] reveal the host's front-line mode of attack against invading *Salmonella* — a previously unknown mechanism and machinery that marks *Salmonella* with the host protein ubiquitin. This tagging sets in motion events that lead to the degradation of the microbial invader.

It was already known that this destruction process involves coating *Salmonella* with ubiquitin[2]. The presence of ubiquitin-tagged *Salmonella* in the cytoplasm launches a defence response when signalling proteins bind to the tagged microbe. This triggers the formation of a double-membraned organelle called an autophagosome, which envelops the bacterium[3,4]. The autophagosome then fuses with the destruction machinery — an organelle called the lysosome[5,6]. Many of the steps in this process are understood, but the contributors to the initial step, the bacterial molecule modified by ubiquitin and the enzyme that directly marks *Salmonella*, were previously unknown.

Ubiquitin is typically joined to target proteins through covalent links to amino (and, in rare cases, hydroxyl) groups on proteins. These ubiquitylation reactions are catalysed by what are termed E3 ligase enzymes. Indeed, many bacterial and host proteins are ubiquitylated during infection by *Salmonella*[7]. Various E3 ligases have been proposed as having a role in combating *Salmonella*[5]. Unexpectedly, however, Otten and colleagues reveal not only that the ubiquitylation of *Salmonella* is catalysed by a different mechanism from that used by a classic E3 ligase, but also that the ubiquitylation target itself is not even a protein.

From microscopy and biochemical studies, the authors gathered evidence pointing to the bacterial target for ubiquitylation as a lipid component of the surface of the bacterial membrane — the molecule lipopolysaccharide (LPS). LPS consists of lipid A in the bacterial outer membrane, sugars and what are termed O-antigen molecules (Fig. 1). Armed with *Salmonella* mutants that had altered versions of LPS, and using super-resolution imaging to track distinct stages of ubiquitylation, the authors analysed the ubiquitylation of *Salmonella* purified from host human cells. They found that these ubiquitylation targets were soluble after boiling. This result is consistent with a ubiquitylation target that is not a protein.

To confirm unequivocally that LPS is the target of ubiquitin tagging, Otten *et al.* used chemical and genetic methods that ruled out amino groups of proteins or other macromolecules as being sites of modification by ubiquitin. Next, using extracts from human host cells as the source of ubiquitylation enzymes, and bacteria from strains with either lipid A alone or shortened forms of LPS on the bacterial surface, the authors reconstituted the ubiquitylation event *in vitro*. This identified lipid A as the minimal form of LPS subject to ubiquitylation, potentially on its hydroxyl groups, its phosphate groups, or both.

This reconstitution of LPS ubiquitylation provided the authors with a way to identify the E3 ligase enzyme. Through skilful biochemical purification, Otten and colleagues identified

the enigmatic enzyme as being the protein RNF213. This large enzyme contains domains of known function (a dynein-like AAA+ motor domain and a zinc-binding RING domain) and others of unknown function[8]. Mutations in the gene encoding RNF213 are associated with a cerebrovascular disorder called moyamoya disease[9]. The deletion of this gene in cells elicits diverse effects — such as ameliorating lipid-associated toxicity or affecting signalling associated with cell death or immune-system function — indicative of RNF213 having multiple functions[10,11].

Otten and colleagues tested versions of RNF213 lacking specific domains or with alterations in known functional amino-acid residues. This demonstrated that the dynein-like AAA+ domain has a role in ubiquitylation, but no obvious such role was found for the RING domain. This is surprising because the RING domain is a hallmark of most E3 ligases, although the authors' finding is consistent with a structural study reporting that the RING domain is not required for RNF213 to ubiquitylate itself[8]. Otten and colleagues identified a molecular motif in RNF213 that is typical of zinc-binding domains of proteins, consisting of four cysteine (C) and two histidine (H) amino-acid residues (CHC_3H) as being required for LPS ubiquitylation *in vitro*.

How does LPS ubiquitylation enable host cells to get rid of *Salmonella*? Previous studies[3,4] indicate that an E3 ligase known as LUBAC assembles what are called linear ubiquitin chains, which form by linking the carboxy terminus of one ubiquitin to the amino terminus of the adjacent ubiquitin in the chain. These chains have dual roles in combating bacteria. They recruit signalling proteins to activate immune responses and they enlist autophagy receptor proteins that promote autophagosome formation.

Given that LUBAC recruitment to bacteria requires a previous ubiquitylation event to initiate linear-chain assembly[3,4], the authors tested whether RNF213 drives this previous event. Indeed, the authors report that cells lacking RNF213, or containing mutations corresponding to the CHC_3H motif of RNF213, failed to recruit LUBAC and to accumulate linear ubiquitin chains (the bacterial target for LUBAC-mediated ubiquitylation remains to be identified). As a consequence of this, these cells also failed to recruit ubiquitin-binding autophagy receptors. These findings confirm RNF213-dependent LPS ubiquitylation as a key initiator of bacterial clearance from host cells.

Although the ubiquitylation of proteins is well established, the transfer of ubiquitin to a lipid is, to our knowledge, unprecedented. This study raises numerous questions for future investigation. Could there be other non-protein ubiquitylation targets, including lipids or carbohydrates? Given that RNF213 is connected to moyamoya disease, it would be worth investigating whether alterations in bacterial ubiquitylation have a role in this poorly understood disorder. Interestingly, Otten *et al.* found that mutations in RNF213 associated with people who have moyamoya disease do not cause a defect in ubiquitylation of bacterial LPS, suggesting that other functional defects — potentially involving unconventional ubiquitylation — cause the disease.

The full details of the mechanism of ubiquitylation that targets LPS remain a mystery. The event involves the protein UBCH7, which typically transfers ubiquitin to a cysteine amino-acid residue on an E3 ligase in preparation for subsequent transfer of that ubiquitin to the target[12]. However, identifying candidate cysteine residues on RNF213 is tricky because the structure of the motif involved in ubiquitylation is not captured in the cryoelectron microscopy structural data[8] for RNF213. It seems likely that RNF213 will not be alone in using unconventional ubiquitylation mechanisms. Precisely how it recognizes LPS, and how the dynein-like AAA+ motor domain contributes to RNF213 function, are interesting questions for the future. The answers might help to determine whether there are other non-protein targets of RNF213. Otten and colleagues' work provides a key step forward in our understanding of the ubiquitin system, and points to yet more secrets waiting to be uncovered.

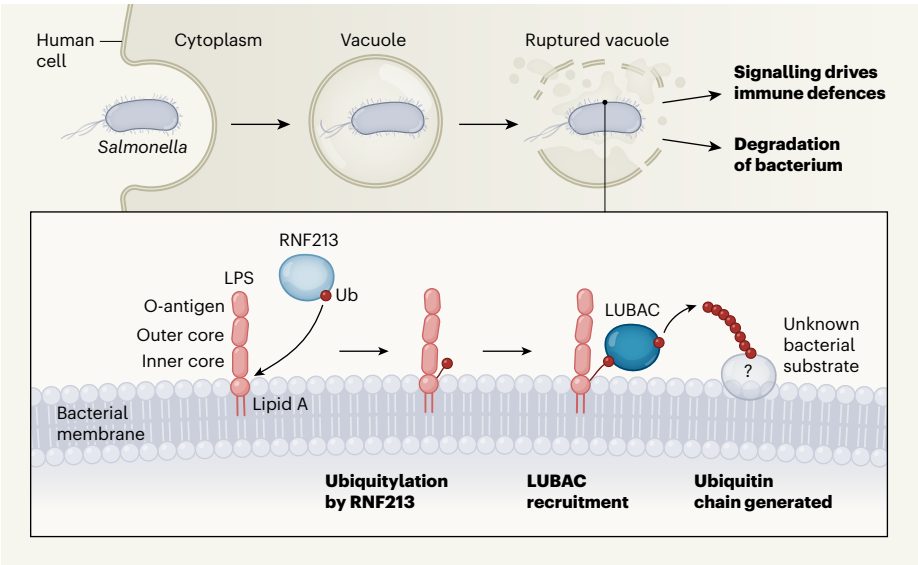

Figure 1 | A human defence response to infection tags a bacterial lipid. A *Salmonella* bacterium can invade human cells, in which it is surrounded by the host membrane in a structure called a vacuole. Vacuole rupture enables the microorganism to access the cytoplasm. Host cells mount a defence response to clear the infection, which relies on tagging the bacterium with the protein ubiquitin (Ub). Otten *et al.*[1] reveal that the initial ubiquitylation event is mediated by the enzyme RNF213 and targets the bacterial lipid lipopolysaccharide (LPS). Until now, proteins rather than lipids have been identified as ubiquitylation targets. LPS consists of lipid A, sugars termed the inner core and outer core, and O-antigen molecules. Otten *et al.* report that RNF213 tags lipid A with ubiquitin. The protein LUBAC, which can also add ubiquitin to targets[3,4], is then recruited and builds a chain of ubiquitin molecules attached to an unidentified bacterial substrate (which might be an unknown protein, possibly one bound to LPS or even the ubiquitin molecule linked to LPS). Formation of a ubiquitin chain triggers signalling that drives defence responses, and the bacterium is degraded.

Brenda A. Schulman is in the Department of Molecular Machines and Signaling, Max Planck Institute of Biochemistry, Martinsried 82152, Germany. **J. Wade Harper** is in the Department of Cell Biology, Harvard Medical School, Boston, Massachusetts 02115, USA.
e-mails: schulman@biochem.mpg.de; wade_harper@hms.harvard.edu

1. Otten, E. G. *et al. Nature* **594**, 111–116 (2021).
2. Perrin, A. J., Jiang, X., Birmingham, C. L., So, N. S. & Brumell, J. H. R. *Curr. Biol.* **14**, 806–811 (2004).
3. van Wijk, S. J. L. *et al. Nature Microbiol.* **2**, 17066 (2017).
4. Noad, J. *et al. Nature Microbiol.* **2**, 17063 (2017).
5. Herhaus, L. & Dikic, I. *Int. J. Med. Microbiol.* **308**, 176–184 (2018).
6. Huang, J. & Brumell, J. H. *Nature Rev. Microbiol.* **12**, 101–114 (2014).
7. Fiskin, E., Bionda, T., Dikic, I. & Behrends, C. *Mol. Cell* **62**, 967–981 (2016).
8. Ahel, J. *et al. eLife* **9**, e56185 (2020).
9. Liu, W. *et al. PLoS ONE* **6**, e22542 (2011).
10. Piccolis, M. *et al. Mol. Cell* **74**, 32–44 (2019).
11. Banh, R. S. *et al. Nature Cell Biol.* **18**, 803–813 (2016).
12. Wenzel, D. M., Lissounov, A., Brzovic, P. S. & Klevit, R. E. *Nature* **474**, 105–108 (2011).

The authors declare competing financial interests: see go.nature.com/2ruajaz for details.

This article was published online on 19 May 2021.