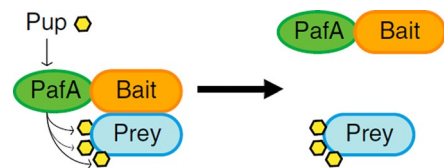


## research highlights

### PROTEIN-PROTEIN INTERACTIONS

#### PUP up the volume

*Nat. Methods* **15**, 715–722 (2018)



Credit: *Nature Methods*

Methods to study protein–protein interactions (PPIs) such as affinity pulldown or proximity-tagging systems are typically more effective for cytosolic PPIs than for those at the membrane. To expand the scope of these methods for the study of membrane PPIs, Liu and Zheng et al. developed the proximity-tagging approach PUP-IT (pupylation-based interaction tagging), which uses the small bacterial protein Pup to label proteins of interest. Analogous to ubiquitination, the Pup ligase PafA phosphorylates the C-terminal Glu of Pup and then conjugates it to a lysine side chain on its target protein. When fused to a protein of interest, PafA ligates Pup to any nearby lysine residues on either the fused protein or its interaction partners. After labeling with Pup–biotin, tagged proteins are enriched and identified by mass spectrometry. The authors applied this method to identify new interaction partners of CD28 and also integrated PUP-IT with the FRB/FKBP dimerization system to label cells expressing certain ligands. PUP-IT can detect weak interactions ( $K_d$  up to 200  $\mu$ M)

in cells, and exhibits high specificity because the activated Pup intermediate does not diffuse from the enzyme. Furthermore, as lysine is present in most human proteins, this approach should be applicable to a wide variety of protein targets. **CD**

<https://doi.org/10.1038/s41589-018-0138-9>

### PLANT DEFENSE

#### Cost-benefit analysis

*Science* **361**, 694–697 (2018)

Maize plants produce secondary metabolites such as benzoxazinoids (BXs), which protect them against insect pests. The western corn rootworm, a specialized maize pest, tolerates and prefers to feed on maize roots with high BX levels. Hu et al. used different maize BX biosynthesis mutants to demonstrate that rootworm larvae prefer roots that produce high amounts of 7-O-methylated, N-hydroxylated BXs such as DIMBOA and DIMBOA-Glc. Surprisingly, BXs themselves were not attractive, but the complexes that form between DIMBOA and iron at the root surface were. The absence of BXs in maize mutants led to iron deficiency in both the plant and the herbivore, suggesting that both require iron–DIMBOA complexes for their development. Finally, the authors identified a DMT-like iron transporter (DvIRT1) required for the rootworm to benefit from maize iron–DIMBOA complexes. Overall, these findings reveal that herbivores can misuse plant micronutrient uptake, which expands the current understanding of plant–herbivore

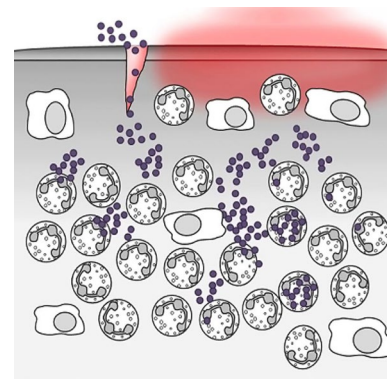
interactions and could inspire new methods for pest management. **GM**

<https://doi.org/10.1038/s41589-018-0139-8>

### INNATE IMMUNITY

#### Fatal chemoattraction

*PLoS Pathog.* **14**, e1007244 (2018)



Credit: *PLoS*

Clearance of skin infections caused by the human pathogenic bacteria MRSA involves production of protective cytokines and chemokines and formation of an abscess composed of neutrophils and extracellular matrix components that encapsulate bacteria to prevent their dissemination to the bloodstream and to other organs. However, the events of the early defense response upon an encounter with the bacteria are poorly understood. Brandt et al. examined LTB<sub>4</sub>, a lipid signaling molecule that has been shown to be produced early upon phagocyte activation, as a potential candidate involved in shaping in the immune response. The authors used mice with deleted 5-LO, an enzyme in the LTB<sub>4</sub> biosynthetic pathway, or those deficient for the high-affinity LTB<sub>4</sub> receptor BLT1 to conclude that LTB<sub>4</sub> is required beginning at the time of infection to promote neutrophil chemotaxis (potentially as a chemoattractant), abscess formation, and thus control of bacterial load. They also defined a novel role for skin-resident macrophages in regulating abscess formation. These results, combined with the observation that an ointment containing LTB<sub>4</sub> can enhance the efficacy of a topical antibiotic against MRSA, suggest that LTB<sub>4</sub> could be useful to boost immunity against MRSA infection. **MB**

<https://doi.org/10.1038/s41589-018-0140-2>

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### GENOME EDITING

#### Diversifying bases

*Nature* **560**, 248–252 (2018)

Directed evolution is an efficient approach to creating biomolecules with desired functions by applying an artificial selection pressure to screen libraries of genetic variants. The degree of diversity in the library can directly affect the screening results. However, diversifying all nucleotides within a defined DNA region in a cell remains a challenge. To overcome this problem and produce libraries with all possible nucleotide mutations, Halperin et al. developed a genomic-editing system called EvolvR by fusing a nicking variant of Cas9 (nCas9) to an error-prone variant of *Escherichia coli* DNA polymerase I (Poll3M), such that mutant nucleotides were introduced downstream of the nicks generated at target sites. By altering the processivity and fidelity of the Poll and the affinity of nCas9 to DNA, the targeted mutation rate could reach as high as 7,770,000-fold greater than that in wild-type cells with a mutagenesis window as long as 350 nucleotides. Using the EvolvR system, the authors identified novel ribosomal mutations in the *E. coli rpsE* gene that conferred spectinomycin resistance. This study provides a targeted mutagenesis tool independent of homology directed repair and adds a new element to the genome-editing toolbox. **YS**

<https://doi.org/10.1038/s41589-018-0141-1>