average abundances of molecules across a population of cells, they were derived using frameworks that account for the inherent randomness of individual reaction events in individual cells. That might seem like a subtle distinction — mathematically accounting for probabilistic mechanisms but then predicting only averages of the resulting statistical distributions. But for most chemical networks, in which reaction rates often depend nonlinearly on concentrations, accounting for probabilistic reactions is necessary even to predict the right averages. Aoki and colleagues' unusual level of rigour in this respect thus makes their results much stronger.

More specifically, the authors focused on a system architecture known as antithetic integral feedback control[2], in which feedback is implemented by actuator and sensor molecules that bind irreversibly to each other (Fig. 1b). If each sensor molecule consistently finds a partner actuator molecule, then the system detects that the output is correctly matching the input. If, instead, there are too many or too few sensor molecules, the actuator molecules automatically adjust the production of sensors to try to get the balance right, like a molecular 'buddy system'. Aoki *et al.* prove mathematically not only that this circuit has the capacity to implement robust perfect adaptation in any chemical-reaction network, but also that all networks that exhibit robust perfect adaptation must at some level embed this kind of antithetic feedback motif.

The authors went on to demonstrate that their theoretical control architecture can be implemented in living cells. They focused on a system that incorporates proteins called σ factors, which regulate the initiation of gene expression in bacteria. Some σ factors are sequestered by binding partners (anti-σ factors), such as the σ factor SigW from the bacterium *Bacillus subtilis* and its anti-σ factor RsiW. The researchers integrated SigW into the model bacterium *Escherichia coli*, and used it to regulate the expression of a green fluorescent protein (GFP) as a reporter of gene expression. They then coupled the activation of the GFP-producing genes to the production of RsiW, which subsequently sequesters SigW. The levels of SigW were also regulated by a small molecule that induces the expression of the *sigW* gene, and which acts as an input to the circuit. If the circuit worked as expected, then the amount of green fluorescence produced by the *E. coli* cells should be proportional to the levels of SigW, and at steady state should be independent of any other parameters.

Sure enough, Aoki *et al.* showed that varying the concentration of the inducer could be used to control GFP output as expected. Yet when the system was disturbed by adding a protease enzyme that degrades both GFP and a protein that affects RsiW production, the fluorescence signal transiently changed but then returned to a level that was indistinguishable from the starting value, demonstrating that the circuit does indeed exhibit robust perfect adaptation. By contrast, in an analogous system that lacked feedback control, the same disturbance systematically lowered the concentration of GFP to about half of its initial value. The authors even replaced GFP with a protein that regulates cell growth, and thereby produced an *E. coli* strain that grew at a constant rate, despite changes in factors that would otherwise alter growth rate.

One possible future direction for such work is to study the circuit in single cells, rather than its average effects across populations. On the one hand, recent work[3] suggests that circuits of this type could increase spontaneous fluctuations, as has also been reported[4] for related classes of reaction scheme. On the other hand, previously published theoretical work[5] from the same research group as that of Aoki *et al.* suggests that more-complex circuit architectures could exhibit robust perfect adaptation without amplifying spontaneous fluctuations. Such behaviour will be necessary to ensure that circuits can perform precise, quantitative functions in any given cell, despite inherent noise and uncertainty. In the same way that reducing error rates in digital circuits was essential for the development of modern computers, the ability to engineer sub-networks of cellular circuits that work precisely and robustly will probably be necessary as we seek to assemble complex synthetic cellular systems comparable to those found in nature. ■

Noah Olsman *and* **Johan Paulsson** *are in the Department of Systems Biology, Blavatnik Institute at Harvard Medical School, Harvard University, Boston, Massachusetts 02115, USA.*
e-mail: johan_paulsson@harvard.edu

1. Aoki, S. K. *et al. Nature* **570**, 533–537 (2019).
2. Briat, C., Gupta, A. & Khammash, M. *Cell Syst.* **2**, 15–26 (2016).
3. Olsman, N., Xiao , F. & Doyle, J. C. *iScience* **14**, 277–291 (2019).
4. Hilfinger, A., Norman, T. M., Vinnicombe, G. & Paulsson, J. *Phys. Rev. Lett.* **116**, 058101 (2016).
5. Briat, C., Gupta, A. & Khammash, M. *J. R. Soc. Interface* **15**, 20180079 (2018).

This article was published online on 19 June 2019.

MICROBIOLOGY

Metabolic mischief as microbes target drugs

Tests of whether a range of gut bacteria can metabolize a diverse group of drugs has revealed that all the microbes metabolized some drugs and that more than half of the drugs were metabolized. SEE ARTICLE P.462

KIM LEWIS & PHILIP STRANDWITZ

All humans are different and, unsurprisingly, also differ in their response to drug treatments. It is usually thought that this variation is due mainly to differences in liver enzymes that specialize in detoxifying ingested molecules. Such enzymes can metabolize drugs, with consequences that include reducing or eliminating drug potency or making them toxic. Understanding how an individual will respond to a given drug is important in developing treatment plans. Yet our knowledge of drug fate in the body is still rudimentary, despite a long history of studies in this area. On page 462, Zimmermann *et al.*[1] put human gut bacteria in the spotlight in the quest to understand how drugs are naturally metabolized.

A handful of previous examples have revealed that the community of microorganisms residing in the gut, termed the gut microbiota, can affect drugs. A classic example is the case of prontosil, the first widely used antibiotic. In the 1930s, the microbiologist Gerhard Domagk found that prontosil could tackle infection by the bacterium *Streptococcus pyogenes* in mice[2]. It was later established that prontosil is metabolized by gut bacteria to generate the molecule sulfanilamide, which is the active form of the drug[3]. Interestingly, had prontosil been tested for activity against *S. pyogenes* in a test tube, as we do today, its capacity to generate an antibiotic would have been missed.

Other examples of gut bacteria affecting drugs include the microbial inactivation of digoxin, which is used for heart conditions[4], and the bacterial modification of the chemotherapeutic agent irinotecan, which causes toxic side effects[5]. Zimmermann and colleagues devised a large-scale approach to tackle the open question of how widespread drug metabolism by the microbiota is.

The authors conducted *in vitro* tests to assess the ability of 76 bacterial strains from the human gut, representing 68 species from the main bacterial taxonomic groupings, to metabolize 271 drugs (Fig. 1). These drugs were chosen to provide a diverse group in terms of factors such as molecular structure or effect on the body. Zimmermann and colleagues

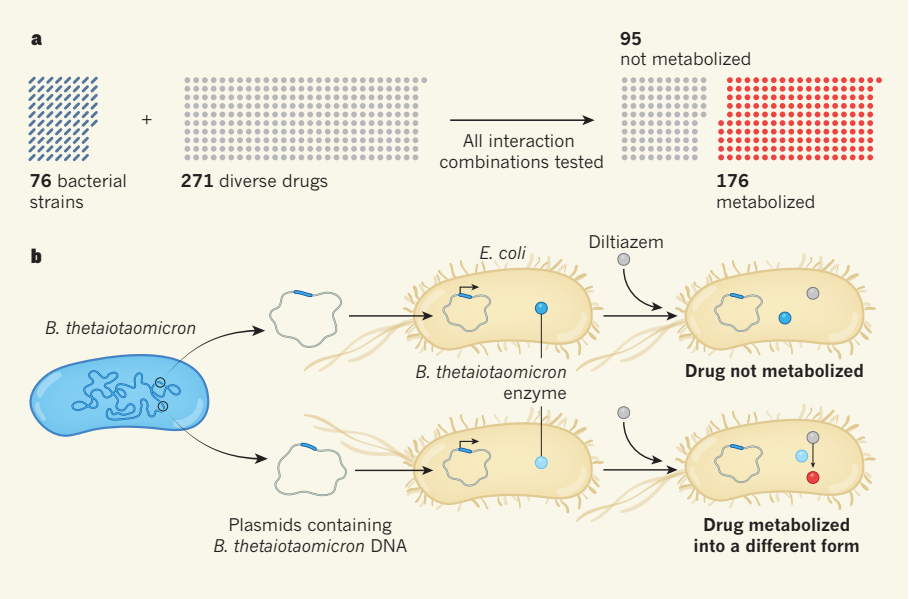

Figure 1 | Studying drug metabolism by gut bacteria. **a**, To assess how commonly drugs are metabolized by bacteria in the human gut, Zimmermann *et al.*[1] tested the ability of 76 bacterial strains (representing 68 species across the main bacterial taxonomic groupings) to metabolize 271 drugs that have diverse structures and functions. This revealed that 65% of the drugs were metabolized — an unexpectedly high number. Some drugs were metabolized into more than one molecular form, and all the bacteria metabolized some of the drugs tested. **b**, To identify some of the bacterial enzymes responsible for drug metabolism, the authors focused on the gut bacterium *Bacteroides thetaiotaomicron*, which metabolized numerous drugs. Zimmermann and colleagues isolated sections of the *B. thetaiotaomicron* genome and inserted them into pieces of circular DNA called plasmids. Plasmids were inserted into the bacterium *Escherichia coli*, which expressed the proteins, such as enzymes, encoded by the *B. thetaiotaomicron* DNA. When these *E. coli* bacteria were exposed to one of the drugs tested, diltiazem, some of the bacteria did not metabolize the drug, but those that did helped to identify the *B. thetaiotaomicron* enzymes responsible for its metabolism.

report that 176 of the drugs tested underwent a substantial metabolic change, caused by least one bacterial strain, that resulted in a reduction in the level of the active drug molecule in bacteria. Each bacterial strain tested metabolized some of the drugs, with the numbers ranging from 11 to 95 drugs per strain. Given that the authors tested a broadly representative panel of drugs, the scale of these results is remarkable because it raises the possibility that most drugs are modified by the microbiota. This type of testing could also be a useful way of singling out drugs that would probably be deactivated by the microbiota.

Zimmermann and colleagues analysed the products of the 176 metabolized drugs using mass spectrometry. This revealed that 868 molecules are derived from these drugs. The numbers indicate that more than one metabolite can be produced from the metabolism of some drugs by gut bacteria. The mass-spectrometry analysis revealed the types of drug modification that occurred, which covered a wide range of chemical alterations, including oxidation, reduction and acetylation (the addition of a C_2H_3O group). The implications of this unexpectedly high diversity of drug alterations will no doubt take researchers a while to address. In the meantime, Zimmermann *et al.* report a few cases of drug metabolism that they examined in detail.

To identify some bacterial enzymes responsible for drug metabolism, the authors chose to profile the gut bacterium *Bacteroides thetaiotaomicron*. This species was a prolific drug metabolizer in their study, modifying 46 of the drugs tested. Zimmermann and colleagues studied how *B. thetaiotaomicron* metabolizes diltiazem, which is used to treat hypertension. The authors engineered *Escherichia coli* bacteria to express sequences from the genome of *B. thetaiotaomicron*, and tested whether the engineered bacteria could metabolize diltiazem. They found that the *B. thetaiotaomicron* gene *bt4096* is required to metabolize the drug.

"Each bacterial strain tested metabolized some of the drugs."

To validate their finding, Zimmermann *et al.* engineered a strain of *B. thetaiotaomicron* that lacked *bt4096*, gave germ-free mice either this strain or wild-type *B. thetaiotaomicron*, and then gave all the animals diltiazem. This confirmed that *bt4096* encodes an enzyme that metabolizes diltiazem. Taking a similar approach, the authors identified genes that are needed to metabolize 18 of the drugs that *B. thetaiotaomicron* can modify.

This type of general strategy should enable the identification of the enzymes in gut bacteria that can metabolize any given clinically used drug or therapeutic molecule in development. Such information would also be useful when testing candidate therapeutics in clinical trials, to try to determine whether a person has gut bacteria that are particularly good at inactivating a particular drug.

Zimmermann and colleagues' study offers a remarkable advance in our understanding of drug dynamics in the body, and will serve as a blueprint for other studies in the fledgling field that seeks to track the effect of microbes on drug metabolism. Yet despite the impressive scope and depth of this analysis, many questions remain, inviting an impatient reader to speculate in the meantime. One issue to consider is that, rather than being taken orally, many drugs are delivered by injection and thus would not be expected to encounter gut bacteria (although some drugs that are delivered by injection can reach the gut and re-emerge in the bloodstream). However, there is a general trend in drug delivery towards oral administration, and advanced methods to facilitate this are in development[6,7]. Over time, there might be a large-scale transition from the use of injected drugs for therapy to more widespread oral delivery. If so, the need to understand the microbiota's role in drug metabolism will become even more urgent.

Drug metabolism by gut bacteria adds to the growing list of ways in which the microbiota can affect the human body. The considerable variation in the microbiota from individual to individual probably also results in variation in drug metabolism. In addition, diet can have a major effect on the composition of the microbiota[8]. Does diet affect the efficiency of drugs by affecting the microbiota? Such issues highlight the complexity of considering a person's microbiota when trying to take a personalized-medicine approach. Adjusting the microbiota to suit our needs, including achieving individually tailored approaches to tackling drug metabolism, is probably where this field is heading. ■

Kim Lewis *is in the Department of Biology, Northeastern University, Boston, Massachusetts 02115, USA.*
Philip Strandwitz *is at Holobiome, Cambridge, Massachusetts 02139, USA.*
e-mail: phil@holobiome.org

1. Zimmermann, M., Zimmermann-Kogadeeva, M., Wegmann, R. & Goodman, A. L. *Nature* **570**, 462–467 (2019).
2. Domagk, G. *Dtsch. Med. Wochenschr.* **61**, 250–253 (1935).
3. Tréfouël, J., Tréfouël, T., Nitti, F. & Bovet, D. *C. R. Soc. Biol.* **120,** 756–758 (1935).
4. Haiser, H. J. *et al. Science* **341**, 295–298 (2013).
5. Guthrie, L., Gupta, S., Daily, J. & Kelly, L. *NPJ Biofilms Microbiomes* **3**, 27 (2017).
6. Abramson, A. *et al. Science* **363**, 611–615 (2019).
7. Banerjee, A. *et al. Proc. Natl Acad. Sci. USA* **115**, 7296–7301 (2018).
8. David, L. A. *et al. Nature* **505**, 559–563 (2014).

This article was published online on 17 June 2019.