MICROBIOME

The microbial pharmacists within us: a metagenomic view of xenobiotic metabolism

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Abstract | Although the importance of human genetic polymorphisms in therapeutic outcomes is well established, the role of our 'second genome' (the microbiome) has been largely overlooked. In this Review, we highlight recent studies that have shed light on the mechanisms that link the human gut microbiome to the efficacy and toxicity of xenobiotics, including drugs, dietary compounds and environmental toxins. Continued progress in this area could enable more precise tools for predicting patient responses and for the development of a new generation of therapeutics based on, or targeted at, the gut microbiome. Indeed, the admirable goal of precision medicine may require us to first understand the microbial pharmacists within.

Microbiome
The combined genetic material and metabolic activities of the microbiota.

Microbiota
The collection of all microorganisms (archaea, bacteria, microscopic fungi, parasites and viruses) found in a given body habitat.

Xenobiotics
Compounds that are foreign to a biological system. For humans, these include drugs, dietary bioactive compounds, food additives and environmental toxins.

Azo bond
A chemical bond composed of N = N.

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Recent advances in the culture-independent investigation of microbial community structure[1,2] and function[2–4], including advances in sequencing technologies and the development of bioinformatic tools, have spawned a veritable 'microbiome renaissance'. Owing to these advances, the microbiota is now often referred to as the 'forgotten organ' because of our understanding and appreciation of its contributions to host physiology, metabolism and disease[5].

The gut microbiota is a diverse and dense microbial community, unparalleled when compared with other body habitats. It is estimated that the gut microbiota is composed of more than 100 trillion cells and 5 million unique genes, outnumbering our own cells and genes[6]. Although the gut microbiota is composed of thousands of species, the majority of them belong to six bacterial phyla: Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Fusobacteria and Verrucomicrobia[7]. In addition to bacteria, the gut microbiota includes microscopic fungi, archaea, protozoa and viruses. The gut microbiota is highly dynamic and demonstrates substantial inter-individual and intra-individual variation. The structure of this microbial community is tightly linked to environmental factors, such as diet and drug intake[3,8] (discussed below), but has also been associated with many additional factors, such as age[9] and host genetics[10].

The microbiota has key functions, such as the breakdown of plant polysaccharides (that is, fibre) that are indigestible to the host, the biosynthesis of essential vitamins and amino acids, the detoxification of xenobiotics, resistance against pathogens and the development of the host immune system. Indeed, the microbiome has now been linked to many areas that were previously considered unrelated to microorganisms, from the circadian rhythm[11] to neuroscience[12–14], cancer biology[15,16], forensics[17,18] and metabolic disease[19,20].

Despite this exponential increase in microbiome research, the links between the microbiota and pharmacology remain largely underexplored. The discovery that microorganisms in the human gut can metabolize drugs dates back nearly a century[21]. Experiments with the sulfonamide antibiotic prontosil, the first broad-spectrum and commercially available antibiotic, demonstrated a lack of antibacterial activity *in vitro*[22]. The discrepancy between the *in vivo* and *in vitro* activities of this drug is due to the fact that gut microorganisms activate prontosil by reducing its azo bond. This biotransformation affects a wide range of compounds, from azo dyes[23], which are commonly used as additives in foods, to sulfasalazine, which is used to treat ulcerative colitis and rheumatoid arthritis[24,25]. Soon after these discoveries, it became apparent that the biotransformation of drugs by the gut microbiota might be far more widespread than previously appreciated, but limited mechanistic insights were uncovered owing, in part, to the difficulties in analysing complex gut microbial communities using traditional culture-based techniques. To date, the fields of pharmacogenetics and pharmacogenomics still largely focus on variations in the human genome, rather than on the genes encoded by the microbiome[26].

Several reviews have highlighted the role of the gut microbiome in pharmacology and precision medicine[27–32]. In this Review, we focus on recent studies that provide insight into the microbial and molecular

Pharmacogenetics
The study of how genetic factors influence therapeutic outcomes.

Pharmacogenomics
The use of sequencing-based genomic methods to analyse the links between genetics and therapeutic outcomes.

Bioavailability
The proportion of an administered compound that reaches systemic circulation and thus has the potential to influence the intended target.

First-pass metabolism
The metabolism of orally ingested compounds before reaching general circulation.

Biliary excretion
The transfer of xenobiotics and other compounds from the plasma to bile through hepatocytes, which is followed by the release of the compounds into the gut lumen.

Enterohepatic circulation
The circulation of xenobiotics and endogenous compounds that are absorbed from the intestines, transported to the liver, and then re-enter the intestine through the bile ducts, where they may be reabsorbed or metabolized by the gut microbiota.

Reduction
A chemical reaction in which the oxidation state of a chemical bond is reduced. For example, a carbon–carbon bond modified to a carbon–hydrogen bond is a reductive transformation.

Hydrolysis
A chemical reaction in which a chemical bond is cleaved using a water molecule, which acts as the nucleophile.

Cytochrome P450 enzymes
(CYPs) A family of enzymes that is responsible for the oxidative biotransformation of xenobiotics and other compounds.

Prodrugs
Drugs that are administered in an inactive form and become active when metabolized.

Folate
A B vitamin that is essential for DNA synthesis, DNA repair and other biological reactions.

mechanisms that are relevant to the prevention and treatment of human disease. Gut microorganisms can affect drug therapy through various different mechanisms that can generally be grouped into direct effects or indirect effects (FIG. 1). Direct mechanisms include the biotransformation of drugs or their metabolites into products with altered bioactivities. Indirect mechanisms involve more complex host–microbial interactions that modulate host pathways for xenobiotic metabolism or transport. We also discuss other classes of xenobiotics, including dietary compounds, food additives and environmental toxins. Finally, we briefly highlight the immediate translational implications of this research and discuss early progress towards microbiome-based diagnostics and co-therapies.

The gut microbiota and pharmaceuticals

The gut microbiota can influence the metabolism of dozens of pharmaceuticals, in many cases changing their efficacy and/or side effect profiles. In this section, we highlight key examples of the direct and indirect mechanisms by which the gut microbiota influences drug therapy.

Microbial metabolism of drugs and their metabolites. The bioavailability of orally administered drugs depends on the extent of first-pass metabolism by intestinal and hepatic enzymes before reaching systemic circulation[33]. However, oral drugs may encounter the gut microbiota before reaching host tissues, representing another important site of first-pass metabolism (FIG. 1). In fact, there is already *in vitro* and/or *in vivo* evidence for the metabolism of 50 drugs by the gut microbiota[28] (Supplementary information S1 (table)). This number is probably an underestimate given the lack of any systematic analyses of the microbial metabolism of drugs in the gut and the vast genetic diversity within the microbiome[34]. Furthermore, the rate of absorption probably has an important role in determining the extent of microbial metabolism owing to the fact that the density of gut microorganisms increases substantially in the distal small intestine (also known as the ileum) and colon. Drugs and their metabolites can also re-encounter the gut microbiota by biliary excretion, at which point they can be further metabolized and reabsorbed through enterohepatic circulation.

Despite the diversity of chemical structures among the drugs that are known to be subject to gut microbial metabolism, two broad chemical transformation patterns have been repeatedly observed — reduction and hydrolysis (FIG. 2; Supplementary information S1 (table)). These two reactions may reflect the energetic demands of the gut microbiota. The gut is largely anaerobic; consequently, microorganisms in the gut cannot rely on oxygen as a terminal electron acceptor for respiration[35]. Reductive xenobiotic metabolism may facilitate anaerobic respiration by increasing the range of alternative electron acceptors that are available for respiration. Conversely, hydrolysis directly provides substrates for microbial growth. For example, many dietary components are glycosylated and their hydrolysis liberates sugars that could be shunted into glycolysis[36].

The commonality of these two reaction types (reduction and hydrolysis) may also imply that there are core microbial species or gene families that affect a wide range of small molecules[37]. If so, the identification of the major players could act as the basis for predicting the manner in which a novel drug will be modified by the gut microbiota. Such knowledge is likely to revolutionize drug development and precision medicine, similar to the advances that followed the discovery that cytochrome P450 enzymes (CYPs) are expressed in the intestine and liver, where they metabolize several xenobiotics[38,39]. Chemical functional groups that are subject to microbial metabolism could be removed through rational design or used to control drug delivery.

The therapeutic effects of several prodrugs that contain azo bonds require bioactivation by gut microorganisms. Following oral administration, the azo bond is reduced by microbial azoreductases, which liberate the biologically active compound. For example, the antibacterial drug prontosil is cleaved by the gut microbiota, which results in the production of triaminobenzene and sulfanilamide[21], a bacteriostatic antibiotic that inhibits folate metabolism. Based on these findings, azo bonds have been used in drug development. For example, sulfasalazine was strategically designed to treat rheumatoid arthritis, which at the time was thought to be the result of bacterial infections, by linking the sulfonamide sulfapyridine with the anti-inflammatory drug salicylic acid by an azo bond[40,41] (FIG. 2). Intact sulfasalazine can be recovered from the stool of antibiotic-treated or germ-free rats, but not in conventionally raised animals[42]. Furthermore, a simplified gut microbiota composed of four bacterial strains (*Bacteroides* sp., *Enterococcus faecalis* and two *Lactobacillus* sp.) is sufficient to restore sulfasalazine metabolism in gnotobiotic rats, and the *in vitro* incubation of sulfasalazine with bacterial isolates from these animals results in drug cleavage[42].

Azoreductases are widespread across several bacterial phyla found in the human gut[28] and possess broad substrate compatibility[43,44]; however, they metabolize azo compounds at different rates depending on the broader chemical structure of the molecule[45]. Gut microorganisms can also metabolize the downstream metabolites of azo reductions. For example, the bioactive component of sulfasalazine, 5-aminosalicylic acid, is inactivated by microbial arylamine *N*-acetyltransferases. The activity of these enzymes can vary up to tenfold between individuals[46], highlighting the considerable inter-individual differences in gut microbial metabolism that may contribute to variations in drug efficacy.

β-glucuronidases are another generalist enzyme family expressed by human-associated gut bacteria that influence the biological activity and toxicity of a wide range of drugs, dietary components and endogenous metabolites[47]. Recent studies have discovered the role of β-glucuronidases in the toxicity of drugs that are used to treat cancer and inflammation. In these examples, gut bacteria metabolize and interfere with drug metabolites that are generated by the host detoxification pathway of glucuronidation. Uridine diphosphate (UDP)-glucuronosyltransferases expressed in the liver add

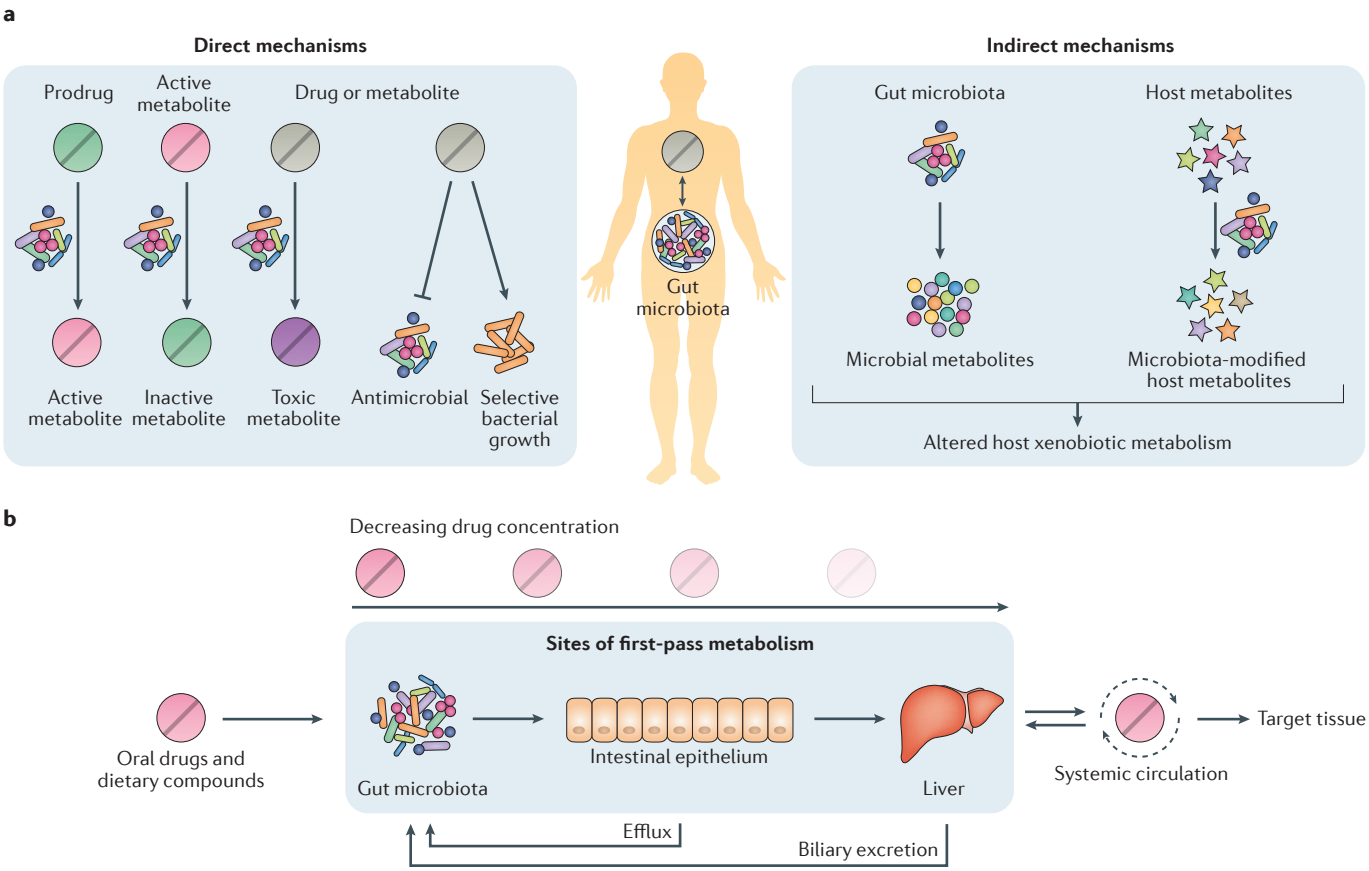

Figure 1 | **Mechanisms that link the gut microbiota and xenobiotic metabolism. a** | The gut microbiota can directly metabolize xenobiotics into active, inactive or toxic metabolites. Xenobiotics may also shape the composition of the gut microbiota through antimicrobial activity or selective growth. The gut microbiota can indirectly influence xenobiotics through the modulation of host pathways that are responsible for metabolism and transport. This can be mediated by microbial metabolites or through the microbial modification of host metabolites. **b** | The gut microbiota is a significant component of first-pass metabolism. Prior to entering systemic circulation and reaching the target tissue, orally ingested compounds are subject to metabolism in the intestine and liver, which decreases the eventual systemic drug concentration. The gut microbiota may metabolize compounds prior to absorption, after efflux from the intestinal epithelium or following biliary excretion from the liver.

Germ-free
Animals devoid of microorganisms.

Gnotobiotic
The colonization of germ-free animals with individual microorganisms or defined microbial communities.

Glucuronidation
The addition of glucuronic acid to a substrate. Glucuronidation is used as a mechanism of xenobiotic metabolism by the host.

Bile acids
Steroid acids produced by the liver that emulsify fats during digestion.

Aglycone
The remaining compound after the removal of a glycosyl moiety.

glucuronic acid to several substrates, including drugs and endogenously produced compounds, such as hormones and bile acids[48]. This biotransformation typically interferes with the biological activity of the substrate and increases the molecular weight and solubility, thereby enabling the elimination of these products in the urine, by renal excretion, or in the faeces, by biliary excretion.

Biliary excretion provides another opportunity for drug metabolism by gut bacterial β-glucuronidases, which can re-activate the drug in the gut causing increased toxicity. An example of this phenomenon is observed with irinotecan (also known as CPT-11), a widely used intravenous prodrug for the treatment of colorectal cancer (CRC). Irinotecan undergoes a complex series of biotransformation events after administration: host carboxylesterases convert irinotecan to the bioactive compound SN-38 (REF. 49); SN-38 is glucuronidated in the liver to the inactive metabolite SN-38 glucuronide (SN-38G); SN-38G is then transported by the biliary route to the intestine where microbial β-glucuronidases liberate the sugar moiety from SN-38G (FIG. 2). The resulting SN-38 in the gut lumen exhibits toxicity towards intestinal epithelial cells and is thought to exacerbate the diarrhoea observed in up to 80% of patients with CRC[50,51]. These side effects can be ameliorated by reducing drug doses or halting drug administration, which in turn impedes effective treatment[52]. A similar mechanism contributes to the side effects of non-steroidal anti-inflammatory drugs (NSAIDs), including diclofenac, indomethacin and ketoprofen[53]. Up to 70% of chronic NSAID users develop injury to the distal small intestine, which is indicated by damage to the mucosa, ulcerations and, in some cases, perforations. In the liver, NSAIDs are subject to host glucuronidation before biliary excretion into the gut lumen. Microbial β-glucuronidases can then liberate the glucuronide, enabling reabsorption of the aglycone into enterocytes. NSAIDs can be further metabolized by these gut epithelial cells into reactive metabolites, which cause mitochondrial and endoplasmic reticulum stress, compromising mucosal integrity and promoting inflammation[51,54].

These examples demonstrate how microbial metabolism can contribute to the side effects of treatment by interfering with host pathways that are involved in drug detoxification. β-glucuronidases are widely distributed

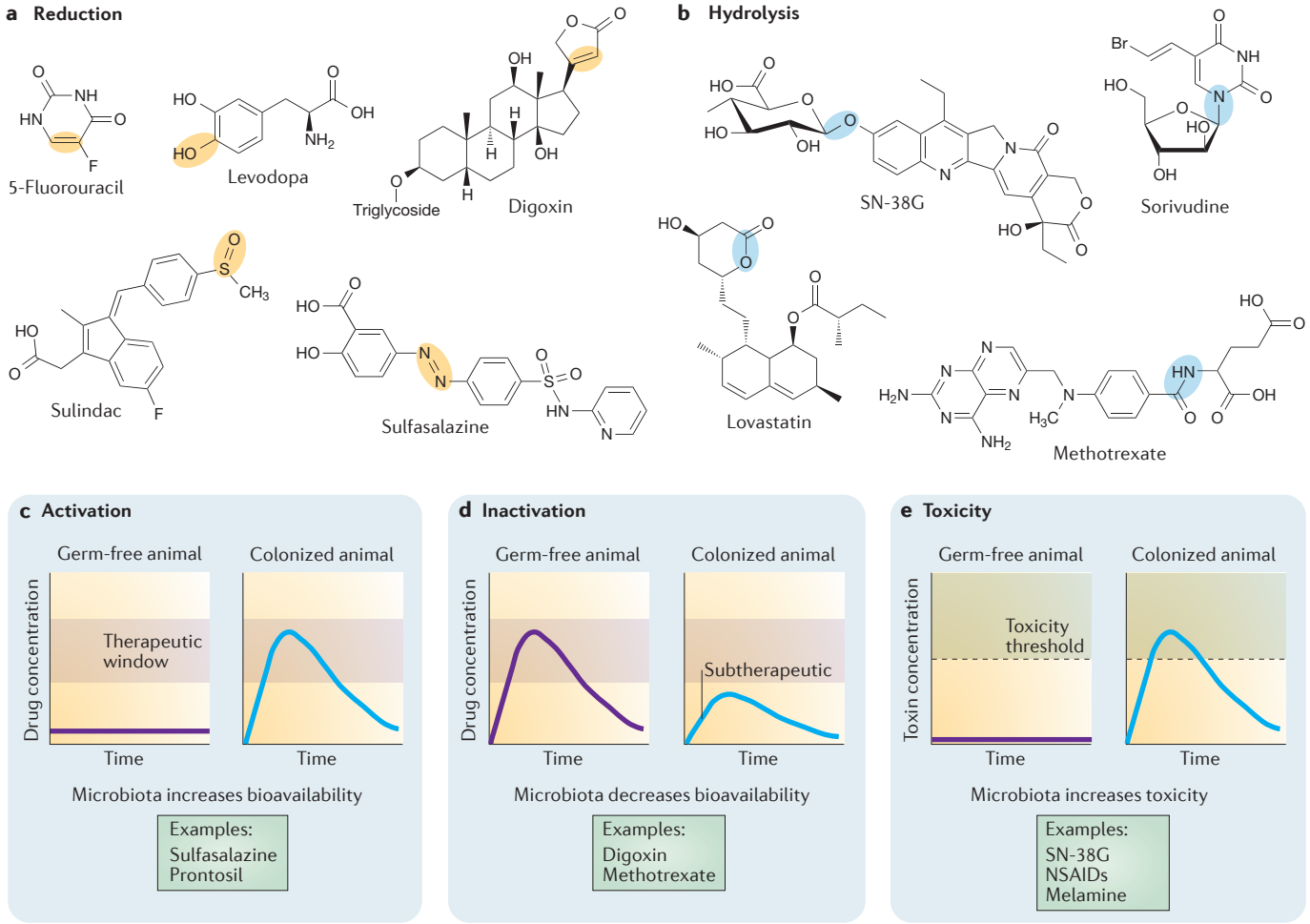

Figure 2 | **Major reaction types catalysed by the gut microbiota and their pharmacological consequences.** The majority of known microbial biotransformations can be segregated into one of two reaction classes: reduction, in which compounds gain electrons from electron donors (part **a**), and hydrolysis, in which chemical bonds are cleaved through the addition of water (part **b**). The sites of modifications are highlighted in orange for reduction reactions and in blue for hydrolysis reactions. For a comprehensive list of drug biotransformations see Supplementary information S1 (Table). The microbial metabolism of pharmaceuticals can lead to their activation (part **c**), inactivation (part **d**) or result in the production of toxic compounds (part **e**); this is illustrated by the differential effects of drugs in germ-free animals, compared with colonized animals. Activation refers to the conversion of a prodrug to its bioactive form, contributing to therapeutic concentrations. Examples of activation include the anti-inflammatory drug sulfasalazine and the antibiotic prontosil. Inactivation refers to the conversion of an active metabolite into a downstream metabolite with reduced bioactivity. Examples of inactivation include the cardiac drug digoxin and the anti-inflammatory drug methotrexate. Toxicity occurs owing to the production of metabolites by microorganisms that are harmful to the host. Examples include the hydrolysis of the anti-cancer drug metabolite SN-38 glucuronide (SN-38G), the hydrolysis of glucuronidated non-steroidal anti-inflammatory drugs (NSAIDs), which are used to treat pain, and the metabolism of the dietary contaminant melamine to cyanuric acid.

across many gut bacterial species, including members of the Proteobacteria, Firmicutes and Actinobacteria phyla[55–60]. However, it remains unclear whether all of these enzymes exhibit a similarly broad substrate scope or whether they are specialized for distinct niches (physical or occupational) in the gastrointestinal tract. Further work is necessary to determine whether the abundance and/or activity of these enzymes can explain inter-individual variations in drug toxicity.

Microbial metabolism can also interfere with the bioavailability of drugs. A classic example of this phenomenon comes from the cardiac glycoside digoxin, which is used for the treatment of cardiac arrhythmia (an irregular heartbeat) and heart failure[26]. The use of digoxin is challenging owing to its exceedingly narrow therapeutic range (0.5–2 ng ml^{-1}), which can make even minor changes to its concentration clinically relevant. Approximately 10% of patients excrete high levels of an inactive metabolite of digoxin, dihydrodigoxin, which results from the reduction of the α,β-unsaturated lactone ring by bacteria[61,62] (FIG. 2). In some cases, more than 50% of the administered drug is inactivated[63], which leads to a substantial decrease in systemic drug concentration. Seminal studies that were conducted in the 1980s

Serum metabolome
The collection of all metabolites found in serum.

suggested that only a single bacterial species, *Eggerthella lenta,* reduces digoxin[64], but unfortunately neither the presence nor the abundance of this species predicts the successful reduction of this drug[64–66]. This discrepancy seems to be driven by strain-level variations in the *E. lenta* population[67]. In *E. lenta* DSM2243, digoxin induces the expression of a two-gene operon, referred to as the cardiac glycoside reductase (*cgr*) operon. The proteins encoded by this operon, Cgr1 and Cgr2, are homologous to enzymes that are involved in electron transport. Cgr1 shows similarity to cytochrome *c* reductases, which are membrane-bound proteins that are involved in shuttling electrons from quinones to an electron reductase partner. Cgr2, which shows similarity to flavin adenine dinucleotide (FAD)-binding fumarate reductases, is predicted to interact and accept electrons from Cgr1 and, in turn, reduce the lactone ring of digoxin[37]. The *cgr* operon is not found in the genomes of *E. lenta* strains that lack the ability to reduce digoxin, which suggests that the *cgr* operon is necessary for the reduction of digoxin and provides an explanation for the difficulty in predicting the reduction of digoxin based only on the presence of *E. lenta* species. Furthermore, the reduction of digoxin is enhanced in the presence of a complex gut microbiota and suppressed by dietary protein[67], which suggests that microbial drug metabolism is sensitive to both microbial and environmental interactions.

Microbial control of xenobiotic metabolism and absorption. Compounds that resist microbial metabolism can still be influenced by the gut microbiota through several mechanisms (BOX 1). Comparisons between germ-free and colonized mice have revealed that the microbiota affects the expression of several host genes that are involved in drug metabolism and transport[68,69]. This influence on gene expression in the host by the gut microbiota can be local[68,70] (in gut tissues) or distant, which includes affecting the most vital organ involved in drug metabolism — the liver[69,71]. In the liver, more than 100 genes are differentially expressed between germ-free and colonized mice[69]. One of the largest groups of differentially expressed genes is the one encoding CYPs. An example that illustrates the importance of the microbiota to xenobiotic metabolism in the liver mediated by CYPs is the anaesthetic pentobarbital (also known as pentobarbitone). Pentobarbital is administered intravenously and is metabolized by CYPs in the liver. Germ-free animals, which show increased expression of CYPs compared with colonized animals, are more efficient at metabolizing pentobarbital than colonized animals[69].

A recent RNA-sequencing-based study confirmed the differential expression of several genes that are involved in xenobiotic metabolism in the liver of germ-free and colonized mice[72]. Furthermore, this study reported a substantial increase in the expression of the xenobiotic-sensing transcription factors aryl hydrocarbon receptor (AhR), constitutive androstane receptor (CAR), peroxisome proliferator-activated receptor-α (PPARα) and nuclear factor erythroid 2-related factor 2 (NRF2) in germ-free mice. However, additional work is necessary to elucidate the mechanisms responsible for these differences in gene expression.

The microbiota also substantially changes the serum metabolome. Comparisons between germ-free and colonized mice revealed that the gut microbiota not only alters the abundance of endogenous metabolites, with

Box 1 | **Microbial modulation of the immune system and drug therapy**

The microbiota has a crucial role in the development and maintenance of the immune system[150]. However, it has only recently become evident that the microbiota helps to mediate the effects of drugs that target the immune system, and that changes to the structure or function of the microbiota represent an unanticipated side effect of treatment.

Several studies have implicated the gut microbiome in the efficacy of drugs that are used to treat cancer. The treatment of mice with cyclophosphamide increased intestinal permeability, promoting the translocation of Gram-positive bacteria into secondary lymphoid organs[151]. This translocation is thought to contribute to the concomitant production of pathogenic T helper 17 (T_H17) cells and memory T_H1 cell immune responses, which are required to limit tumour growth[151,152]. Consistent with this model, the efficacy of cyclophosphamide was reduced in germ-free mice and in animals that were treated with broad-spectrum antibiotics, whereas the adoptive transfer of pathogenic T_H17 cells restored cyclophosphamide efficacy. A similar dependence on the gut microbiota was found for CpG-oligonucleotide immunotherapy[153]. The response to CpG-oligonucleotides in germ-free mice and in animals treated with broad-spectrum antibiotics was poor, as evidenced by a decrease in cytokine production and tumour necrosis. Oxaliplatin, a platinum-based drug that induces apoptosis through the production of reactive oxygen species in the tumour[154], was also dependent on the microbiota[153]. More recently, members of the *Bifidobacterium* genus were shown to enhance the immune response to tumours in a manner that increased the efficacy of anti-programmed death ligand 1 (α-PDL1), an antibody that blocks immune inhibitory pathways[155]. Also, cytotoxic T lymphocyte protein 4 (CTLA4) blockade immunotherapy was shown to depend on particular *Bacteroides* species (*Bacteroides thetaiotaomicron* and *Bacteroides fragilis*)[156]. Together, these results indicate that the immune response to specific members of the gut microbiota may help set the stage for cancer treatment.

Conversely, recent studies have suggested that anti-inflammatory drugs that are used to treat inflammatory bowel disease may affect the gut microbiome. The ulcerative colitis-like phenotype of TRUC mice (deficient in T-bet (*Tbx21*) and recombination activating gene 2 *(Rag2)*) is dependent on the cytokine tumour necrosis factor (TNF)[157]. Treatment with blocking antibodies against TNF suppresses colitis in patients[158]. This therapy is also effective in the TRUC model and is accompanied by a substantial increase in the abundance of the *Staphyloccocus* genus[159]. However, more work is necessary to determine the functional consequences of the changes to the gut microbiota in response to anti-TNF blocking antibodies and other related therapies.

10% of shared metabolites differing in abundance by at least 50%, but also contributes unique microbial compounds to systemic circulation[73,74]. Some of these microbial metabolites are processed by the host in a manner analogous to xenobiotics (that is, conjugation)[74]. This overlap between the host response to drugs and microbial metabolites may have implications during drug therapy; for example, it may result in increased toxicity or half-life of xenobiotics owing to competition between the drug and microbial metabolites for the same host enzymes that are involved in drug detoxification or elimination. An example of this type of interaction can be observed with acetaminophen (also known as paracetamol), which is one of the most widely used drugs worldwide. Overdose of acetaminophen can lead to severe and sometimes fatal hepatotoxicity[75], and both drug metabolism and toxicity vary between individuals[76,77]. Acetaminophen is metabolized in the liver and the predominant metabolites that result, acetaminophen sulfate and acetaminophen glucuronide, are inactive. However, a minor metabolite, *N*-acetyl-*p*-benzoquinone imine (NAPQI), causes toxicity in the liver. Based on these findings, a metabolomic study aimed to determine whether pre-dose urinary metabolite profiles could predict acetaminophen metabolism in humans[78]. Pre-dose levels of *p*-cresol sulfate were found to be inversely associated with the ratio of acetaminophen sulfate to acetaminophen glucuronide. The microbial metabolite *p*-cresol is an end-product of tyrosine and phenylalanine metabolism and has been demonstrated to be produced by several microorganisms, including those belonging to the Firmicutes (*Clostridium difficile*), Bacteroidetes, Actinobacteria and Fusobacteria phyla[79,80]. Following absorption and circulation, *p*-cresol is metabolized in the liver to *p*-cresol sulfate. *p*-cresol and acetaminophen are both substrates of the human cytosolic sulfotransferase 1A1 (SULT1A1)[81] (FIG. 3). This competition probably impedes the ability of the host to detoxify acetaminophen, which increases the likelihood of accumulating the toxic metabolite NAPQI.

Host–microbial interactions may also influence drug efficacy. Statins, which are cholesterol-lowering drugs prescribed for coronary artery disease, have substantial inter-individual variations in efficacy, with up to 33% of patients failing to reach lipid-lowering targets[82]. In humans, the response to treatment with simvastatin (indicated by low-density lipoprotein (LDL) cholesterol levels) is positively associated with the pre-treatment levels of three secondary bile acids produced by the microbiota: lithocholic acid, taurolithocholic acid and glycolithocholic acid[83]. Although the mechanism, or mechanisms, responsible for this association remains unknown, one intriguing hypothesis is that primary bile acids may compete for the same intestinal transporters that enable the absorption of statins (FIG. 3). Therefore, microbial bile acid metabolism may decrease this competition, priming the host for more effective statin therapy.

A similar type of interaction may influence the efficacy of tempol, an antioxidant that protects against diet-induced obesity in animal models[84]. Treatment of mice with tempol altered the relative abundance of the two dominant bacterial phyla in the distal gut, increasing the abundance of Bacteroidetes and decreasing the abundance of Firmicutes[85]. In the Firmicutes phylum, members of the genus *Lactobacillus* were substantially reduced (FIG. 3). Several members of the *Lactobacillus* genus encode bile salt hydrolases, which produce free bile acids by deconjugating taurine-conjugated bile acids[86]. Consistent with these findings, tempol increases the intestinal concentration of several taurine-conjugated bile acids, including tauro-β-muricholic acid (Tβ-MCA). Tβ-MCA is an antagonist of the farnesoid X receptor (FXR), a master regulator of lipid, glucose and bile acid metabolism[85,87,88]. Tempol does not further reduce adiposity in intestinal-specific *FXR*-null mice[85], consistent with the hypothesis that changes in microbial bile acid metabolism and subsequent signalling by the FXR pathway contribute to the mechanism of action of tempol. It remains to be determined whether tempol has direct antimicrobial activity against members of the gut microbiota or whether the observed changes in microbial community structure are mediated through drug interactions with the host.

The gut microbiota and dietary xenobiotics

In addition to influencing drugs, the gut microbiota can metabolize numerous xenobiotic compounds found in our diet, including natural products and chemical additives. In some cases, these compounds have beneficial health effects that depend on microbial bioactivation. In other instances, the gut microbiota can produce toxic metabolites. In this section we highlight key examples of how gut microbial metabolism affects the health effects of the foods that we consume.

Diet-derived bioactive compounds. Our diet is rich in small molecules that have important consequences for human health and disease. Many of these 'diet-derived bioactive compounds' are metabolized by the gut microbiota (FIG. 4; Supplementary information S2 (table); Supplementary information S3 (table)) and some are dependent on this transformation for activation and/or absorption[47]. Below, we highlight examples with considerable recent evidence elucidating their interaction with, and dependence on, the gut microbiota.

Clinical and epidemiological studies suggest that dietary polyphenols, such as anthocyanins (ACNs) and proanthocyanidins (PACs), protect against metabolic syndrome[89,90]. Supplementation of high-fat diets with ACNs or PACs has been shown to suppress the expression of genes that are involved in fatty acid and triacylglycerol synthesis, the regulation of lipogenesis and cholesterol biosynthesis, and the assembly of very low-density lipoproteins[90–92]. ACNs and PACs have also been argued to stop the development of insulin resistance by increasing insulin signalling, glycogen accumulation and the secretion of adiponectin in the presence of free fatty acids[93]. Intriguingly, studies in rodents indicate that just 6–12% of radiolabeled polyphenols are metabolized and enter circulation during their passage through the gut[94,95], which raises the question of how these compounds confer their protective effects. Recent studies on polyphenol extracts

Conjugation
The addition of a chemical unit (for example, glucuronic acid or glutathione) to xenobiotics, increasing the solubility and molecular weight of the parent compound and facilitating elimination from the body.

Metabolic syndrome
A collection of physiological and biochemical conditions, defined as a combination of high blood pressure, increased blood sugar levels, excess fat and abnormal cholesterol levels. This syndrome increases the risk of heart disease, stroke and diabetes.

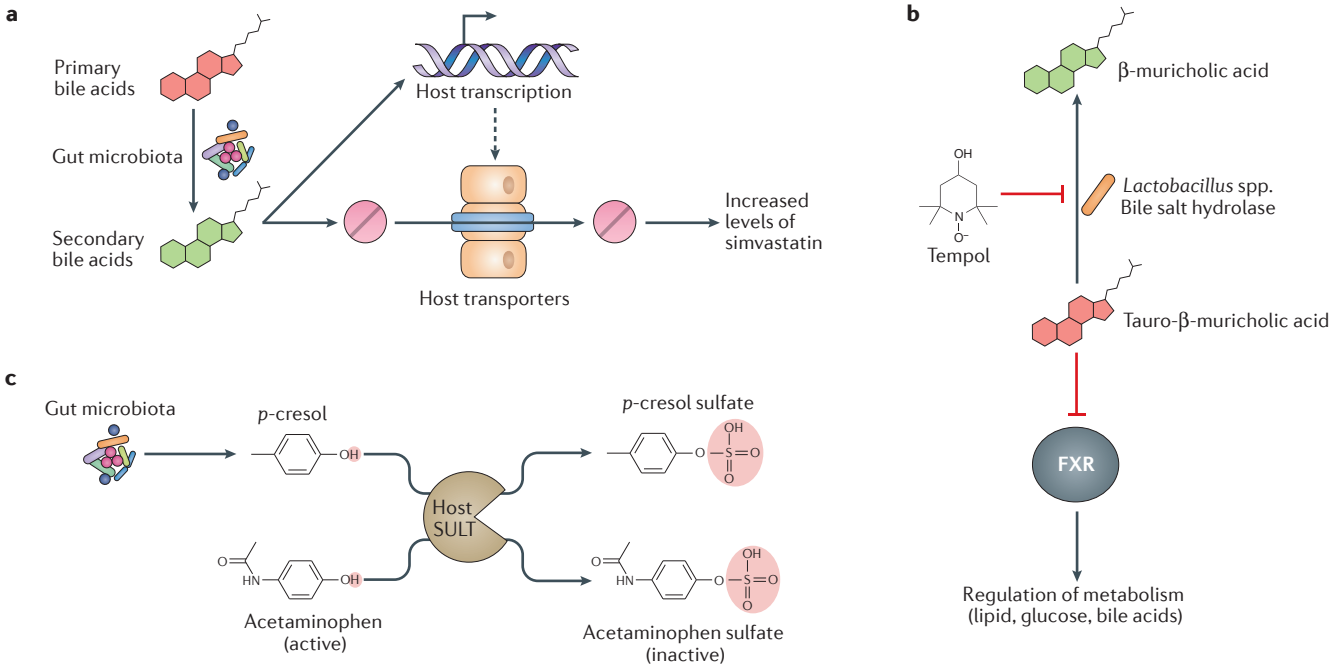

Figure 3 | **Host–microbiota interactions shape therapeutic outcomes. a** | Levels of the drug simvastatin in the host positively correlate with levels of secondary bile acids. The metabolism of bile acids by gut bacteria possibly contributes to the absorption of simvastatin by modulating the expression of host transporters or by directly competing with host transporters. **b** | The protective effects of tempol on diet-induced obesity are mediated through the gut microbiota. Treatment with tempol reduces the abundance of *Lactobacillus* spp., which are involved in deconjugating taurine-conjugated bile acids into free bile acids through the action of bile salt hydrolases. A reduction in the abundance of *Lactobacillus* spp. results in increased levels of taurine-conjugated bile acids, such as tauro-β-muricholic acid, a known antagonist of the metabolic regulator farnesoid X receptor (FXR). **c** | Microbial metabolites compete with drugs for host xenobiotic metabolism enzymes. The microbial product *p*-cresol, a product of tyrosine metabolism, and acetaminophen both act as substrates for the same enzyme, the host sulfotransferase SULT1A1. Therefore, increased levels of *p*-cresol inhibit the conversion of acetaminophen (the active form) to acetaminophen sulfate (the inactive form).

isolated from grapes[20] and cranberries[96] provide support for a mechanism that acts through the gut microbiota. Mice fed high-fat diets that were supplemented with polyphenols showed a reduction in diet-induced weight gain and adiposity, improved insulin sensitivity, and diminished markers of intestinal inflammation and oxidative stress compared with controls[20,96]. These improvements were coupled with substantial sevenfold-to-tenfold blooms of *Akkermansia muciniphila*, a mucin-degrading bacterium that is argued to have an important role in the preservation of the integrity of the gut mucus layer, thus limiting the risk of systemic inflammation[97]. The abundance of *A. muciniphila* has been linked to reduced weight gain, adiposity, insulin resistance, and/or inflammatory markers in many contexts, including during pregnancy[98], following gastric bypass surgery[19,99], in prebiotic or metformin treatment experiments[100,101], and in other polyphenol feeding experiments involving green or black tea[102,103] or a grape juice and red wine mixture[103]. Furthermore, the administration of live (but not heat-killed) *A. muciniphila* was sufficient to reduce host adiposity, inflammatory markers and insulin resistance in diet-induced obese mice[97,101]. Further work is required to establish how polyphenols promote the growth of *A. muciniphila* and whether this effect is direct or mediated through changes in host physiology. However, a recent *in vitro* study reported that the exposure of a complex human faecal microbial community to black tea or grape-derived polyphenols can directly increase the abundance of *A. muciniphila*, which suggests a limited dependence on host factors in this process[103].

Fruit-derived ellagitannins are thought to provide protective properties for the plant by preventing microbial decay[104]. The hydrolysis of ellagitannins releases ellagic acid, which can be metabolized by the gut microbiota into several structurally related urolithins that can reach high concentrations locally, in the colon, and systemically[105]. Several *in vitro* studies have shown that urolithins have antioxidant, anticancer, anti-inflammatory and antimicrobial properties; however, there are currently a limited number of *in vivo* and mechanistic studies on urolithins. A recent study found that ellagic acid metabolism varied substantially between individuals but could be generally grouped into three categories depending on the metabolites generated, including a subset of individuals that did not produce any urolithins[106,107]. This implies that the composition of the gut microbiota in an individual is a key determinant of whether beneficial products in a diet can be extracted or activated. Bacterial isolates from the gut that are capable of metabolizing ellagitannins have been identified, including members of the *Gordonibacter* genus in the Actinobacteria phylum[108,109].

Metformin
An oral medication used to treat type 2 diabetes.

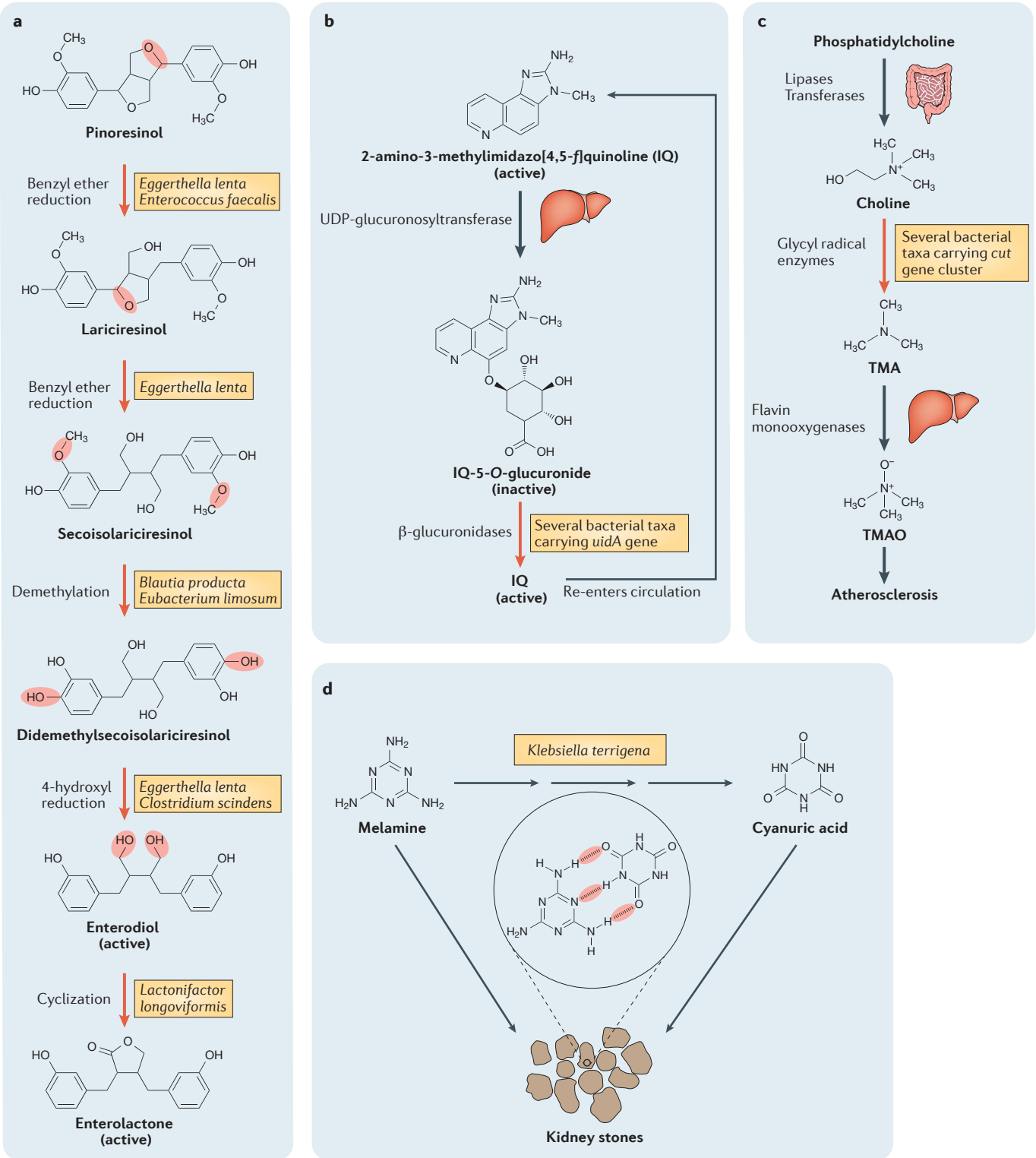

Figure 4 | **Microbial metabolism of dietary compounds. a** | The plant-derived dietary lignans pinoresinol and secoisolariciresinol are metabolized by several bacteria into the cancer-protective compounds enterodiol and enterolactone. **b** | The microbiota is responsible for the reactivation of the heterocyclic amine 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) after hepatic inactivation, which leads to the delayed excretion of the carcinogenic compound. **c** | The microbial production of trimethylamine (TMA) from choline-containing compounds represents a crucial link between dietary phosphatidylcholine and the atherosclerotic metabolite trimethylamine *N*-oxide (TMAO). **d** | The metabolism of melamine by the gut microbiota leads to kidney stones. *Klebsiella terrigena* converts melamine to cyanuric acid, which complexes with melamine to form insoluble aggregates in the kidney. *uidA*, β-glucuronidase. *cut*, choline utilization cluster; UDP, uridine diphosphate.

Two phytoestrogen classes, isoflavones and lignans, represent plant-derived chemicals that are metabolized by a diverse range of gut bacteria, such as members of the Actinobacteria, Bacteroidetes and Firmicutes phyla, to molecules that bind to oestrogen receptors and may elicit protective effects against breast cancer[110–112]. One such isoflavone, daidzin, is a glycosidic isoflavone that is predominantly found in soy products and is metabolized to equol by several species of bacteria residing in the gut (for example, *Enterococcus faecium*, *Lactobacillus mucosae*, *Bifidobacterium* sp. and *Eggerthella* sp.) by glycosidic cleavage and reduction of an α,β-unsaturated ketone[113]. Facile absorption introduces equol into systemic circulation in which it demonstrates a high affinity for oestrogen receptor-β (ERβ). The biological effect of the affinity of equols for ERβ may be particularly apparent in Asian populations, who traditionally consume diets that are rich in phytoestrogens, with the consumption of approximately 10 mg of isoflavones per day being associated with a lower incidence of breast cancer (risk reduced by 12%)[114]. This may be attributed to both a higher concentration of isoflavones (equol precursors) in the gut and the presence of gut bacteria that are able to generate equol[115]. By contrast, women in western populations, who typically consume a much smaller quantity of isoflavones (approximately 0.3 mg per day), demonstrated no association between isoflavone consumption and breast cancer risk. Although there are results that both support[116] and discount[117] that individuals producing equol may have a decreased risk of developing breast cancer, further consideration and characterization of the equol-producing bacteria that are present in the microbiota will help to explain these results.

The protective effects against breast cancer that are associated with the consumption of plant lignans (found in flaxseed, sesame seeds, legumes, grains, berries, cruciferous vegetables and tea) are similarly dependent on metabolism by bacteria in the gut[118,119]. In a multistep pathway involving several gut bacteria (including *E. faecalis, E. lenta*, *Blautia producta*, *Eubacterium limosum*, *Clostridium scindens* and *Lactonifactor longoviformis*), lignans, such as pinoresinol and secoisolariciresinol, are metabolized to the bioactive 'mammalian lignans' enterodiol and enterolactone[120] (FIG. 4). The protective effects of enterodiol and enterolactone in a chemically induced breast cancer model were assessed when germ-free rats or germ-free rats that had been colonized with a bacterial consortium that was demonstrated to convert secoisolariciresinol to enterodiol and enterolactone (composed of *Clostridium saccharogumia*, *E. lenta*, *B. producta* and *L. longoviformis*) were fed a flaxseed-rich diet[121]. For the colonized group, the number of breast tumours was 2.5 times lower and the tumour size and weight were ~2 times lower than was observed for the germ-free group. These findings highlight the importance of gut bacteria for actualizing the protective effects of lignans against breast cancer.

Conversely, microbial biotransformation may exacerbate the effect of harmful compounds that are derived from the diet. For example, the activity of microbial β-glucuronidases may contribute to associations between the risk of CRC and the intake of heterocyclic amines, which are compounds formed during the charring of meat. Several carcinogenic heterocyclic amines are detoxified through hepatic glucuronidation, including prevalent compounds that are derived from the diet, such as 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) and 2-amino-3,8-dimethylimidazo[4,5-*f*]-quinoxaline (MeIQx)[122] (FIG. 4). The glucuronidated compounds are excreted into the intestinal lumen through bile, at which point microbial β-glucuronidases could theoretically release the conjugate group, reactivating the toxic compound and thereby augmenting its genotoxicity. To this end, studies on IQ have repeatedly observed more DNA damage and DNA adducts in conventional mice than in germ-free mice[123,124]. Importantly, the genotoxicity of IQ was assessed directly in gnotobiotic rats that were monossociated with isogenic *Escherichia coli* strains either carrying or deficient in the gene *uidA*, which encodes β-glucuronidase[125]. The *E. coli* β-glucuronidase increased the colonic genotoxicity of IQ threefold and led to several peaks in urinary and faecal excretion of this compound, consistent with enterohepatic circulation.

In addition, analysis of a large clinical cohort recently found an association between the risk of cardiovascular disease and microbial metabolites of choline-containing compounds, which are liberated in the intestine through the breakdown of dietary phosphatidylcholine mediated by lipases. In the colon, choline-containing compounds undergo metabolism by microbial glycyl radical enzymes[126] to form the intermediate gas trimethylamine (TMA; FIG. 4). In turn, TMA is absorbed and oxidized by hepatic flavin monooxygenases to form TMA *N*-oxide (TMAO), a metabolite linked to the accumulation of cholesterol in macrophages and foam cell deposition[127], and a higher risk of a major adverse cardiac event[128]. A similar mechanism seems to be responsible for the link between atherosclerosis and dietary L-carnitine, a compound abundant in red meat[129]. The microbial choline utilization (*cut*) gene clusters that are responsible for the production of TMA have been detected in 20 members of the human gut microbiota, including representatives of the major Firmicutes, Proteobacteria, and Actinobacteria (but not Bacteroidetes) phyla[126]. As the capability to utilize choline is unevenly distributed across the gut microbiota, inter-individual differences in gut microbial community composition could potentially act as biomarkers for the strength of the linkage between diet and cardiovascular outcomes.

Artificial sweeteners and emulsifiers. Many processed foods contain chemical additives that are meant to enhance flavour or maximize shelf-life without any consequence for the consumer. However, several recent studies have begun to suggest that these dietary additives may have deleterious interactions with the gut microbiota.

Non-caloric artificial sweeteners (NAS) are widely used food additives that are designed to be resistant to host metabolism and provide a sweet flavour without increasing caloric intake. However, in some cases, the gut microbiota is still capable of modifying these

compounds, converting them to bioactive metabolites of which cyclamate is a classic example. Many intestinal microorganisms, including those belonging to the genera *Enterococcus*, *Clostridium*, *Corynebacterium, Campylobacter* and *Escherichia*, can convert cyclamate to cyclohexylamine[130], which has exhibited toxicity in animals. A small number of animals that were dosed with high levels of cyclamate developed bladder tumours and, as a result, this compound has been banned from being included in any food or drug in the US and UK since 1970 (REF. 131). Recent studies suggest that several other NAS alter the gut microbiota, including xylitol[132] and saccharin[133]. Chronic consumption of NAS in mice was shown to affect gut microbial community structure[133], resulting in an increase in the abundance of bacteria belonging to the *Bacteroides* genus and members of the Clostridiales order. These differences seem to have a functional consequence, as germ-free mice that are colonized with gut microorganisms from NAS-treated mice or that are colonized with faecal microorganisms that were exposed to NAS *ex vivo* have impaired glucose homeostasis. Preliminary results in humans suggest that saccharin may only affect a subset of individuals[133], which potentially explains why large-scale epidemiological analyses have failed to link the consumption of NAS to diabetes[134]. Further work is required to determine the mechanisms through which NAS shapes the structure and function of the gut microbiota, and whether these changes have implications for glucose homeostasis in the host.

The gut microbiota may also be affected by emulsifying agents. These additives are used in processed foods, such as ice cream, to enable them to be stored for long periods of time without particles falling out of suspension. However, these compounds have detergent-like properties and may have an effect on the composition of the gut microbiota and on the integrity of host tissue. Controlled feeding of two emulsifying agents, carboxymethylcellulose and polysorbate-80 (Tween 80), to mice resulted in a reduction in the thickness of intestinal mucus and, as a result, microbial cells showed increased encroachment towards epithelial cells[135]. The composition of the gut microbiota was also affected, with a decrease in the abundance of Bacteroidales (an order in the Bacteroidetes phylum) and an increase in the abundance of mucolytic bacteria, such as *Ruminococcus gnavus*[135]. Although the overall mucus layer decreases in thickness in treated animals, the increase in the abundance of mucolytic bacteria may reflect increased accessibility to mucin by bacterial penetration. These shifts in microbial community structure were accompanied by low-grade inflammation, increased gut permeability, increased weight and adiposity, and the development of metabolic syndrome. Interestingly, emulsifiers failed to have this effect on germ-free mice. However, the transfer of the microbiota from animals that were treated with emulsifiers to germ-free recipients was sufficient to induce the same symptoms, even in the absence of further emulsifier feeding. This suggests that the composition of the gut microbiota is a key driver of metabolic syndrome and low-grade inflammation. Furthermore, inflammation may be exacerbated among individuals that are predisposed to intestinal conditions, such as colitis, as feeding emulsifiers to genetically sensitized animals that are prone to inflammation (interleukin-10 (*Il-10*)-deficient and Toll-like receptor 5 (*Tlr5*)-deficient mice) promoted a colitis phenotype.

Toxicity caused by dietary contaminants. The industrial compound melamine and its microbial metabolite cyanuric acid form an insoluble complex that interferes with kidney function, which can lead to severe renal toxicity[136]. In China in 2008, milk that was contaminated with melamine caused more than 50,000 infant hospitalizations and six deaths due to renal failure[137], provoking scientific inquiry into the mechanisms responsible. Administration of melamine in combination with broad-spectrum antibiotics resulted in decreased kidney damage in animal models[138], potentially due to the decreased microbial conversion of melamine to cyanuric acid[139,140]. The colonization of animals that were fed melamine with *Klebsiella terrigena*, which produces cyanuric acid, led to increased kidney damage[138] (FIG. 4). Interestingly, *K. terrigena* is sparsely distributed in the gut of healthy individuals, being present in approximately 1% of the population[141]. Therefore, additional work is necessary to determine whether the gut microbiota is a major contributor to inter-individual differences in the toxicity of melamine and other dietary contaminants.

Moving towards microbiome-based medicine

The emerging appreciation that the gut microbiota influences pharmacology and nutrition has begun to reveal the immediate translational potential of this research[28,30,142]. Continued progress in this area could lead to approaches to improve drug outcomes by altering the gut microbiota, and to predict drug outcomes by metabolite or genetic screening of the gut microbiota. In this section, we highlight recent studies that provide a proof-of-principle demonstration for each of these goals (FIG. 5). Furthermore, these studies may also provide additional information about the microbiome that can be used to harvest new drugs (BOX 2).

Targeting the microbiome for therapeutic benefit. Despite the numerous undesirable biotransformations that are catalysed by the gut microbiota, our ability to manipulate gut microbial metabolism in a targeted fashion to prevent these biotransformations remains in its infancy. One approach would be to develop small-molecule inhibitors that target the microbial enzymes that are responsible for undesirable xenobiotic transformations (FIG. 5). However, the complexity of the gut microbiota and its many redundant enzymes raises questions about whether these targets will truly be druggable. The answer seems to be yes for the bacterial β-glucuronidases, for which several inhibitors now exist that have minimal effects on the mammalian homologue[143–146]. This specificity towards the bacterial enzymes was revealed with the assistance of crystallography and bioinformatics, which demonstrated that these inhibitors interact with a 'bacterial loop' that is highly conserved and well distributed across the

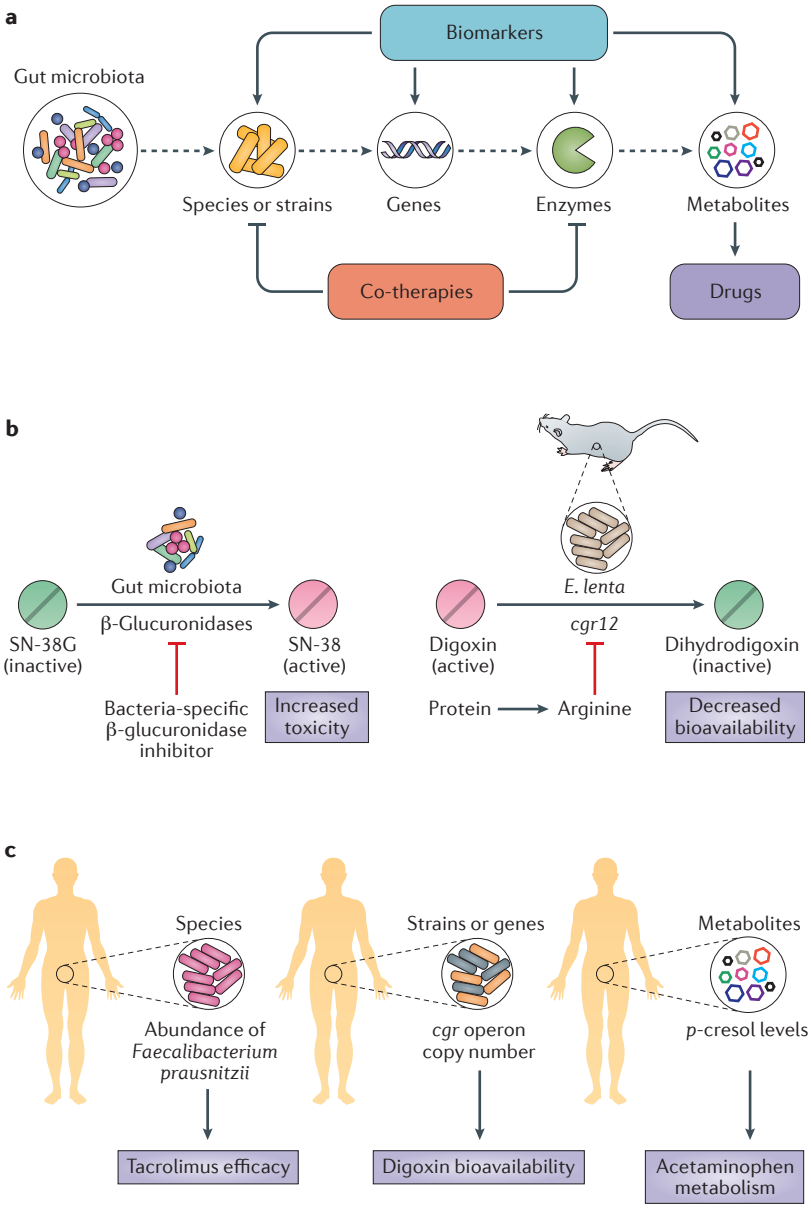

Figure 5 | **Translational implications of microbiome research in pharmacology.** **a** | Metagenomic and metabolomic approaches enable the dissection of microbial communities at several scales from complex communities to individual metabolites. This information can be used to find biomarkers, to develop co-therapies that target the microbiota or to identify novel drugs. **b** | Inhibiting microbial enzymes in the gut. Such examples include using small molecules to inhibit the activity of bacterial β-glucuronidases (left panel) and the dietary inhibition of cardiac drug inactivation by *Eggerthella lenta* (right panel). Bacterial β-glucuronidases convert the compound SN-38 glucuronide (SN-38G) to SN-38, which results in increased toxicity. Therefore, inhibiting bacterial enzymatic activity can lower toxicity. Strains of *E. lenta* that express the cardiac glycoside reductase (*cgr*) operon can catalyse the transformation of digoxin into the inactive metabolite dihydrodigoxin. However, the microbial conversion of digoxin is inhibited by feeding mice a high-protein diet, which restores the activity of digoxin. **c** | Microbiome-based diagnostics. Microbial species, strains, genes, enzymes or metabolites could also be used for diagnostics. For example, measuring the abundance of specific bacterial species, such as *Faecalibacterium prausnitzii*, could be used to predict the efficacy of tacrolimus. Similarly, the microbiota could be analysed for the presence of genes that are associated with the bioavailability of digoxin, such as the *cgr* operon. The levels of specific microbial metabolites, such as *p*-cresol (which is associated with acetaminophen metabolism), could also be assessed.

enzymes in gut bacteria, but is absent from the mammalian enzyme[146]. However, not all bacterial glucuronidases contain this loop, including those from members of the Bacteroidetes phylum[60].

Notably, β-glucuronidase inhibitors are capable of rescuing mice from drug toxicity. Mice receiving irinotecan along with a β-glucuronidase inhibitor had a substantially lower incidence of diarrhoea and less damage to the gastrointestinal epithelium than mice receiving irinotecan alone[146] (FIG. 5). Similarly, in animals exposed to the NSAIDs diclofenac, indomethacin and ketoprofen, co-administration of the β-glucuronidase inhibitor reduced mucosal injury and enteropathy compared with control mice that were not receiving the inhibitor[54,147]. These inhibitors may also be useful for minimizing the toxicity that is induced by other bacterial deglucuronidation events, such as the colonic reactivation of the heterocyclic amine IQ[125].

Dietary intervention represents an alternative strategy to control the microbial biotransformation of drugs (FIG. 5), as diet has been shown to rapidly and reproducibly alter the gut microbiota in humans and animal models[8,148]. Research into the cardiac drug digoxin has provided an initial proof-of-principle for this approach. The amino acid arginine prevents digoxin inactivation by *E. lenta in vitro*, decreasing the expression and activity of the genes responsible (the *cgr* operon)[67]. In these experiments, germ-free mice were colonized with *E. lenta* and fed a similar diet that only differed in the amount of total protein. Following the administration of digoxin, mice that were fed a high-protein diet showed substantially increased serum and urinary digoxin levels compared with controls. Furthermore, dietary protein did not have an effect on mice colonized with a strain of *E. lenta* that lacks the *cgr* operon[67]. Therefore, these results highlight the potential to revise the nutritional guidelines for drugs based on their interaction with the gut microbiota.

Developing microbiome-based diagnostics. Another emerging area of interest is the development of diagnostic biomarkers that predict the optimal drug or dosage based on the gut microbiome (FIG. 5). Although this could theoretically be used for any microbial metabolite, species or gene family linked to the drug responsible (or even in an unbiased fashion), we highlight three examples of more targeted tests: the pain-reliever acetaminophen, the cardiac drug digoxin and the immunosuppressant tacrolimus.

As discussed above, the pre-dose levels of *p*-cresol sulfate were found to be inversely associated with the ratio of acetaminophen sulfate to acetaminophen glucuronide. Therefore, it has been suggested that the concentration of *p*-cresol sulfate could act as a predictive biomarker for drug detoxification, helping to minimize liver damage[78] (FIG. 5).

Similarly, the variation in metabolic activity between distinct strains of *E. lenta* suggests a potential microbiome-based genetic test for drug bioavailability. Strains of this species vary as to whether they carry the genes responsible for digoxin reduction — the *cgr*

Box 2 | **Mining the microbiome for new drugs**

In addition to influencing drug outcomes, the gut microbiome may provide a rich source of novel therapeutics. A recent analysis of 2,430 reference genomes from human-associated microorganisms identified more than 14,000 biosynthetic gene clusters that are predicted to synthesize diverse small molecules from saccharides, non-ribosomally encoded peptides, polyketides, and ribosomally encoded and post-translationally modified peptides[160]. The gut and oral cavity represented the richest sources of gene clusters, with considerable variation in the number of gene clusters between individuals. Of note, gene clusters that encode antibacterial thiopeptides were found at every body site. A new class of thiopeptide, named lactocillin, was isolated from the vaginal isolate *Lactobacillus gasseri* JV-V03. Interestingly, lactocillin had broad activity against Gram-positive pathogens, consistent with the activity observed for other thiopeptides, but lactocillin had no activity against other vaginal *Lactobacillus* isolates[160].

Bile acid metabolizing bacteria, or their metabolites, could represent another source of new drugs. Broad-spectrum antibiotics that are used in clinical practice can provide an opportunity for infection by enteric pathogens[161]. In patients who are undergoing bone marrow transplantation and in mice exposed to a panel of antibiotics, the abundance of *Clostridium scindens* was inversely associated with infection with *Clostridium difficile*[162]. *C. scindens* was sufficient to protect mice from infection following antibiotic treatment owing to its unique ability to generate the secondary bile acids deoxycholate and lithocholate, which inhibit the growth of *C. difficile*[162–164]. Therefore, bacteria that are able to metabolize bile acids, or the metabolites that result from these reactions, could represent a novel treatment regimen for infection with *C. difficile*.

Pharmacopoeia
A manual for the preparation and use of medicinal drugs. The name is derived from the Greek words *pharmakon* (drug) and *-poios* (making).

operon[67]. Using quantitative PCR, human faecal samples were evaluated for their *cgr* ratio (the proportion of *cgr* abundance normalized to the abundance of the *E. lenta* species). Notably, the *cgr* ratio could be used to effectively discriminate between microbial communities exhibiting low digoxin reduction versus high digoxin reduction (FIG. 5). Such diagnostics might enable physicians to distinguish a priori which patients are likely to respond favourably to digoxin therapy.

The immunosuppressant drug tacrolimus has a very narrow therapeutic range and a small number of patients who receive this therapy require an increase in dosing. A study examining kidney transplant recipients found a positive correlation between recipients who required an increase in tacrolimus dosing and the abundance of the gut bacterium *Faecalibacterium prausnitzii*[149] (FIG. 5). Although the reason for the observed correlation between *F. prausnitzii* and tacrolimus dosing is unknown, the abundance of this bacterium may still act as a useful biomarker for increased dosing requirements.

Finally, an alternative approach may be to identify surrogate biomarkers (for example, proteins, metabolites or nucleic acids) in the blood or urine that predict the abundance of specific strains of *F. prausnitzii, E. lenta* or other clinically relevant microorganisms, which would enable the rapid and routine stratification of patients according to their predicted therapeutic outcomes.

Outlook

The studies discussed throughout this Review emphasize that the human gut microbiome has an important role in xenobiotic metabolism, influencing the efficacy and toxicity of drugs, dietary compounds and environmental toxins. Gut microorganisms have evolved numerous enzymes that enable them to directly metabolize xenobiotics and their metabolites, as well as ill-defined mechanisms for controlling host xenobiotic metabolism and transport.

Given the recent resurgence in microbiome research, it is now an opportune time to consider a more comprehensive view of pharmacology that includes the membership, structure and function of our resident microbial communities and a deeper understanding of their interactions with each other, their host habitat and the nutritional milieu of the gastrointestinal tract. Continued progress will require concerted efforts to expand the scope of metagenomic and metabolomic studies, while also developing complementary experimental and computational approaches to model gut microbial metabolism along the entire length of the gastrointestinal tract. This work will provide fundamental insights into poorly studied, but clinically relevant, microbial taxa and enable the more complete annotation of the genetic dark matter of the microbiome. Studies on xenobiotic metabolism, and microbial metabolism in general, will be essential for the microbiome field to move beyond simply describing 'who is there' to interpreting 'what they are doing'. The translational implications of this work are already becoming apparent, whether through the discovery of gut microbial signatures that predict drug outcomes, co-therapies that precisely target members of the gut microbiota or new drugs harvested from the microbiome (BOX 2). Together, these results emphasize that the microbiome will be a key component of a twenty-first century pharmacopoeia, as it provides a modifier, target and source for the drugs of the future.

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Competing interests statement
The authors declare competing interests: see Web version for details.

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