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Redox metabolism of ingested arsenic: Integrated activities of microbiome and host on toxicological outcomes

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Abstract

Arsenic is a human carcinogen and is linked to diverse pathologies in many organ systems. Arsenic exposure primarily occurs by ingestion of inorganic arsenic (i*As*). Post-ingestion metabolism of i*As* involves sequential cellular transformations by the microbiome and then by the host. Methylation is particularly critical for detoxification in both bacterial and mammalian cells, and is performed by a conserved mechanism in both phyla that involves alternating oxidations and reductions of the arsenic. Methylation-independent redox state modifications, glutathionylation, and thiolation reactions can also occur both in the gut-lumen and in host cells and, in combination, these can result in host cells being exposed to diverse inorganic and organic arsenicals with vastly different toxicological impacts. The toxicity of arsenate (As^V) stems from it being a phosphate analog whereas inorganic arsenite (iAs^{III}) and trivalent-methylated arsenic toxicities stem from their being electrophiles that bind to reactive cysteines or selenocysteines, in particular in the active sites of critical redox-active enzymes. In general, toxicities of inorganic or organic pentavalent forms are lower than those of the corresponding trivalent compounds. Metabolism of arsenic in either prokaryotes or eukaryotes has been studied in depth; however, few studies have assessed the interplay between the two. In particular, little is known about how metabolism by the microbiome impacts host exposure, metabolism, and outcomes. Understanding microbiome-host arsenic-interactions will foster development of novel targeted strategies to relieve or prevent arsenic-associated pathologies.

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1. Arsenic exposure

Arsenic (*As*) is a Group 1 human carcinogen that causes skin, lung, and bladder cancer [1]. Non-cancerous adverse health effects include skin lesions, diabetes, neuropathies, cardiovascular disease, and respiratory/reproductive dysfunction [2,3]. Humans are primarily exposed through drinking water, although exposure *via* food, soil, tobacco use, and air also occur [4,5]. The World Health Organization has set a safety limit for arsenic in drinking water at 10 μg/L (10 ppb), yet drinking water of an estimated 200 million people globally exceeds this [2]. Arsenic in water largely comes from natural weathering of rock and soil, but agricultural and industrial practices can be important [6]. Once arsenic enters an ecosystem, it is cycled by environmental microorganisms and is not easily cleared, resulting in persistent contamination [7–9].

2. Arsenic metabolism and toxicity

Drinking water contains mostly inorganic pentavalent (arsenate, iAs^V) or trivalent (arsenite, iAs^{III}) arsenicals. iAs^V is a phosphate analog that enters cells *via* phosphate transporters, and iAs^V toxicity is due to interference with cellular kinases, phosphatases, or oxidative phosphorylation. In contrast, iAs^{III}, which is more toxic than iAs^V, enters cells through distinct transporters (see below) and is a strong electrophile, whose toxicity stems primarily from disruptive interactions with nucleophilic thiols or selenols in the active sites of critical cellular enzymes [10–12].

Both microbes and mammals use methylation to detoxify arsenic. This biotransformation is catalyzed by As^{III}-*S*-adenosylmethionine (SAM) methyltransferases, which are designated as ArsM in bacteria and AS3MT in

mammals. The entry substrate for the enzyme is As^{III} and the reaction mechanism likely involves a series of oxidation-linked methylation steps that generate As^{V} products, each of which are followed by a 2-electron reduction to the As^{III} form (Figure 1) [13]. Reducing power after each oxidative methylation is provided by either the thioredoxin (Trx)- or glutathione (GSH)-systems. The ArsM or AS3MT reactions generate methylated arsenicals in both the trivalent and pentavalent states: monomethylarsonic acid (MMA^{V}), monomethylarsonous acid (MMA^{III}), dimethylarsinic acid (DMA^{V}), dimethylarsinous acid (DMA^{III}), trimethylarsine, and trimethylarsine oxide [14]. The trivalent methylated species, MMA^{III} and DMA^{III}, are substantially more toxic than either their pentavalent or inorganic counterparts yet, overall, methylation promotes excretion [15,16] (see below).

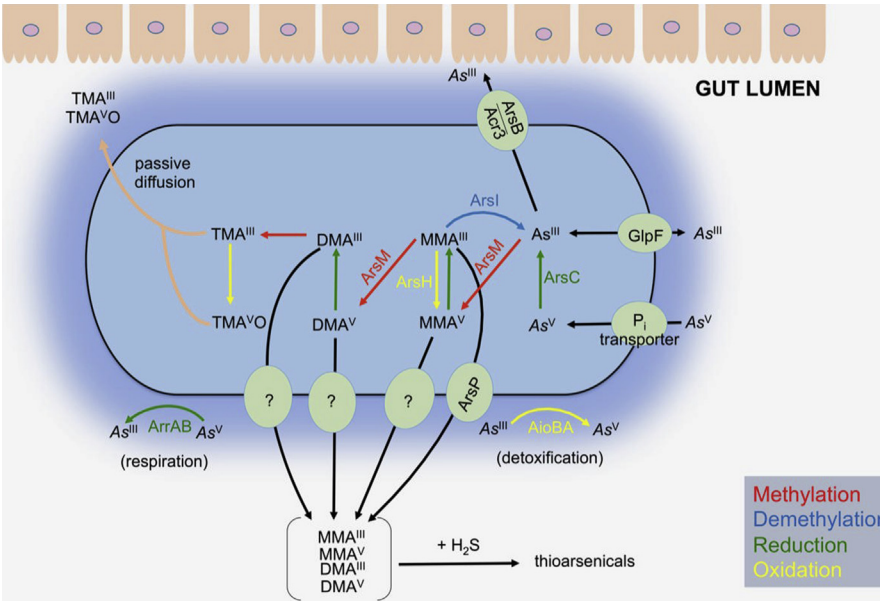
Arsenic biotransformation and transport mechanisms are similar in bacteria and mammalian cells, yet there are some important differences and their respective contributions to host exposure outcomes may differ. Arsenite species entering mammalian cells rapidly become conjugated to GSH (glutathionylated) in reactions likely catalyzed by glutathione-transferases (GST; Section 4.4) [17–19]. GSH also participates in microbial detoxification mechanisms, although more studies are needed on this (Section 3) [20]. More recently the formation and toxicity of thioarsenicals, which contain arsenic-sulfur bonds, have become of interest, as these compounds have been detected in both microbes and animals [21] (Figure 1).

Likely because most arsenic exposure is through ingestion, few studies have investigated the impact of arsenic on microbiomes other than within the gut, such as the skin or lung microbiomes. Dermal contact, in particular, is not a major source of exposure [22]. Studies on non-smoking arsenic-exposed subjects did not show significant changes in the lung microbiome [23]; however, there were differences between the low and moderate exposure groups that likely warrant further study. In the predominant case of oral exposure, it has been shown that arsenic can dramatically alter the microbiome of the gut [24]. The following sections will overview arsenic metabolism by the gut microbiota and the host. We will overview arsenic-interactions with cellular redox systems and discuss the implications of these processes on toxicological outcomes of i*As* ingestion.

3. Arsenic interactions with gut microbiota

Microbially driven arsenic chemical transformations include reductions, oxidations, glutathionylation, methylation, de-methylation, and thiolation. Importantly, the microbiome is heterogeneous due to both deterministic and stochastic factors, and so outcomes of exposure may vary both within and between individuals. Likewise, alteration of the microbiome can alter arsenic exposures, as recently reviewed [25]. Here, we focus

Figure 1



Possible microbiome-driven arsenic transformations in the gut. Methylation (red), demethylation (blue), reduction (green), and oxidation (yellow). Transformations that are not color-coded are either non-catalytic or have not been specifically linked to a microbial enzyme. Both uptake and efflux pathways are shown (black arrows). Uptake of As^{V} is mediated by phosphate transporters (Pit and Pst) whereas As^{III} enters through the glycerol uptake facilitator protein (GlpF). As^{III} efflux is mediated by ArsB or Acr3 and MMA^{III} efflux is mediated by ArsP.

more on microbiome and host redox metabolism that can potentially influence exposure outcomes.

Few studies have been performed on microbiome-arsenic interactions, but it is anticipated that many reactions observed in environmental microbes could occur in the human gut, as many relevant genes have been identified. Bacteria transform the redox status of arsenic for either detoxification or energy conservation. One means of detoxification is *via* the GSH/glutaredoxin (GSH/Grx)-driven reductase, ArsC, which converts As^{V} to As^{III}, for active export by the As^{III} efflux transporters, ArsB or Acr3 [12,26]. Another means of bacterial detoxification is to oxidize As^{III} to As^{V} with the *aioBA*-encoded As^{III} oxidase, and some anaerobes couple this oxidation with nitrate reduction as a means of energy production [27–29]. In another energy yielding transformation, some bacteria transfer electrons directly to As^{V} (i.e. respiration) with the surface-anchored reductase, ArrBA, thereby reducing As^{V} to As^{III} (Figure 1) [30]. Importantly, the respiratory As^{V} reductase, ArrBA, is quite different from the detoxifying reductase, ArsC, perhaps evolving in parallel to capitalize on an energy-conserving metabolism in energy limiting environments.

Detoxification *via* As^{V} reduction is somewhat paradoxical, since As^{III} is more toxic. Yet, this is an ancient mechanism with origins prior to the divergence of bacteria, archaea, and eukaryotes [14] and thus is likely to have arisen in anoxic (reducing) environments on early earth. Under present (oxic) conditions, the toxicity of iAs^{III} production is presumably offset by promoting more effective export. Why evolution favored this pathway as opposed to simply exporting As^{V} may also be due to the chemical similarity between phosphate and iAs^{V}, and the downstream consequences of inadvertently exporting valuable phosphate. Regardless, co-deletion of *arsC* and *arsB* leads to significant arsenic sensitivity [31], indicating that As^{V} reduction is important for detoxification.

Arsenicals are detoxified both by microbial and mammalian methylations [14,32,33]. However, some microbiome methylations could actually increase host toxicity. For example, trivalent mono- and di-methylarsenicals (MMA^{III} and DMA^{III}) can be oxidized to their pentavalent counterparts *via* ArsH to decrease their toxicity [34], while other bacteria export the highly toxic MMA^{III} *via* ArsP, which could increase host toxicity (Figure 1) [35]. Detoxification of MMA^{III} can also be achieved by ArsI, a C-*As* bond lyase, producing the less toxic, pentavalent MMA^{V} [36]. Although the gut lumen is generally considered a reducing environment that would favor $As^{V} \rightarrow As^{III}$ transformations, oxygen levels are potentially high enough along the intestinal epithelium to allow oxidative arsenic transformations [37].

In addition to the above enzyme-driven reactions, it is now appreciated that non-enzymatic byproducts of microbial metabolism and respiration also influence arsenic chemistry. For example, hydrogen sulfide (H_2S) produced by sulfate-reducing bacteria can react with arsenicals to form more toxic thioarsenicals, such as dimethylmonothioarsinic acid ($DMMTA^{V}$) [38]. Thioarsenicals have also been identified in the gut after inorganic or methylated arsenic exposure [39], and they likely have distinct transport, metabolic, and toxicologic activities, many of which are in need of further investigation [40,41].

A recent bioinformatic search for the As^{V}-reducing operon *arsABCDR* found widespread distribution of this pathway among human gut bacteria [37]. However not all isolates of a given species contained the pathway, implying that As^{V} reduction is not a universal trait and that only a subset of the gut microbiome is relevant to arsenic metabolism. Arsenic exposure studies in mice support this idea, as the composition of the microbiome changes dramatically during acute high-level arsenic exposure [24]. Based on this, we hypothesize that bacteria with the potential to either detoxify or otherwise resist arsenic will be enriched during exposure, which in turn enhances their metabolic impact on the host.

Future studies need to address which arsenic metabolism pathways are favored in the microbiome and their net impacts on host health. For example, rapid reduction of As^{V} to As^{III} was observed in both active and sterilized (control) *in vitro* cultures of human stool [42], suggesting that enzymatic reduction by the microbiome is not required for this transformation. Conversely, methylated and thiolated arsenicals were only generated in the active stool cultures, suggesting that these species were microbiome-dependent. In mice, methylated and thiolated arsenicals are produced in the cecum following exposure to As^{V} [39]. These studies support the microbiome's role in arsenical speciation changes following ingestion. Although no study to date has directly addressed the overall influence of microbiome-driven arsenic metabolism on a mammalian host, it is becoming appreciated that the gut microbiome must be considered as an important factor in risk assessments for arsenic exposure [25].

4. Arsenic interactions with host metabolic processes

4.1. Overview

Following transformations within the microbiome, ingested arsenic will interact sequentially with (i) enterocytes; (ii) endothelial cells; (iii) blood cells; (iv) hepatocytes; and (v) peripheral organs. Along this journey, the impacts and outcomes of arsenic exposure will be influenced by cellular import; interactions, metabolism, and conjugation reactions inside of these cells; and cellular export. In each case, there will be differential effects dependent both on the chemical

species of arsenic involved and the cellular components present in that cell type.

4.2. Arsenic transport into cells

Arsenic exits the intestinal lumen and enters host circulation *via* both transcellular (i.e. transporter-mediated through enterocytes) and paracellular (i.e., past cell-cell junctions between enterocytes) routes. The specific route used depends on the arsenic species present which, in turn, depends on the species ingested and on microbiome metabolism (Section 3). Within the transcellular route, the similar physical-chemical properties of As^{V} and phosphate allow uptake of As^{V} and perhaps thiolated-As^{V} species by sodium/phosphate co-transporters (Na^{+}/P_i-IIb; SLC34A2) at the apical membrane of intestinal epithelium [43–45]. SLC34A2 might also transport thiolated-As^{V} species [45]. Candiate. As^{III} uptake transporters in the gut include aquaglyceroporins (AQPs), glucose transporters (GLUTs), and organic ion transporters (OATPs) [46–49]. Once in the circulation, arsenic can interact with serum proteins and, indeed, a high-affinity As^{III}-binding site on human serum albumin has been defined biochemically [50]. Cellular uptake and efflux of arsenic by endothelial cells along blood vessels remains incompletely characterized. However, endothelial cells are sensitive to arsenic toxicity [51] and they express AQPs and glucose transporter-1 (GLUT1), which are likely candidate proteins for import and efflux of arsenic [52,53]. Hepatic uptake of arsenicals likely occurs through AQP9, GLUTs, and OATPs, which are expressed in liver [44]. Little is known about urinary excretion transport pathways; however predictions were recently summarized [44].

4.3. Arsenic interactions with cellular components

As^{V}, as a phosphate analog, has little direct interaction with cellular redox systems [10]. By contrast, As^{III} exerts its toxicity largely through interactions with Cys-thiols or, in rare but important situations, with selenocysteine (Sec)-selenols [10]. Sec, which is similar to Cys but much more reactive, is found in only 25 human proteins (selenoproteins); nearly all are oxidoreductases in which Sec functions in the active site [54]. These include all three Trx-reductases (cytosolic TrxR1, mitochondrial TrxR2, and testis-specific TrxR3), five of the six glutathione peroxidases (Gpx), and a Met-sulfoxide reductase, making Sec a critical component of the disulfide reductase-based antioxidant systems (Section 4.5) [55]. The interactions of arsenites with specific Cys or Sec residues is dependent on reactivities, concentrations, and accessibility.

Although the thiol of GSH is only modestly reactive [56], the high cytosolic concentration of GSH (~5 mM) [57] makes it the predominant molecule to react with As^{III} entering cells, resulting in mono-, di- and tri-glutathionylated oxo- and thio-arsenical species [58,59]. Lipoic acid is a non-abundant but highly reactive dithiol-containing metabolite that is also bound by As^{III}. Lipoic acid is a critical cofactor in the citric acid cycle [60] and, indeed, As^{III}-dependent inactivation of the citric acid cycle and some other metabolic enzymes is through interactions with a bound lipoic acid cofactor [10]. By contrast, little has been reported about As^{III} interacting with other thiol-metabolites, such as Cys, homocysteine (Hcy), or coenzyme-A (CoA), which is probably due to their relatively low reactivities and concentrations.

Protein thiols are also abundant in cells but most have modest reactivity or accessibility [61]. Some data suggests that proteins with multiple solvent-exposed Cys residues (e.g., Cys4 or His1-Cys3 Zn-finger transcription factors), are higher affinity As^{III}-binders *in vitro* than are similar proteins with fewer exposed Cys residues, such as His2-Cys2 Zn-fingers [10]. Interestingly, mammalian metallothioneins (MT), which are small Cys-rich chelators that sequester various potentially toxic metals and metalloids, will also bind trivalent arsenicals *in vitro* [62] but have not been captured in arsenic-based affinity screens [63]. Moreover, a recent study indicated arsenic did not bind MT1 in As^{III}-treated rat livers [64]. Because As^{III} is rapidly glutathionylated in cells, we suspect that, *in vivo*, the abundant but relatively non-reactive thiols on MTs and many other proteins cannot displace GSH from As^{III}. Rather, the predominant protein-targets of As^{III} have highly nucleophilic Cys or Sec residues in their enzyme active sites. This includes many components of cellular antioxidant systems and in some species (e.g., rat but not human), hemoglobin [10]. Studies using affinity purification approaches on protein extracts or affinity pull-down approaches to capture As^{III}-bound proteins from living cells have yielded disulfide reductase system components including Trx1; peroxiredoxins; protein-disulfide isomerase-3; GSTs; glutathione reductase (Gsr); and the Gpx- and TrxR-families of selenoproteins [10,63]. In addition to targeting these highly nucleophilic active site residues, trivalent arsenic exposure can exert influences through targeting other Cys residues in proteins. For example, As^{III} is known to interact with specific reactive Cys residues on some redox-responsive regulatory proteins, such as on the Kelch-like ECH-associated protein-1 (Keap1), which regulates the dominant cytoprotective transcription regulator nuclear factor erythroid-derived 2-like 2 (Nrf2). Arsenic binding by Keap1 influences the Keap1-Nrf2 interaction, leading to activation of Nrf2 and a strong cytoprotective transcriptional response [65].

4.4. Arsenic metabolism in mammalian cells

Besides glutathionylation, the primary mammalian cell biotransformation is AS3MT-catalyzed methylation [58,66] (Figure 1; see above). AS3MT preferentially uses tri- glutathionylated-As^{III} ($As(GS)_3$) as a substrate and liberates the 3GSHs [14,67], although As^{III}-protein-

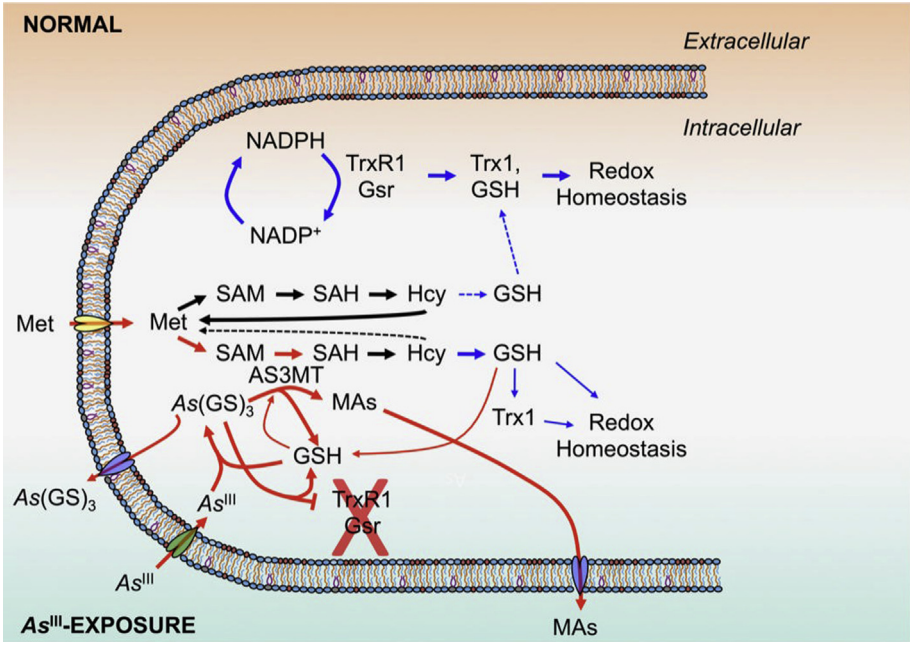
conjugates can also serve as substrates [41]. The AS3MT reaction consumes methyl (CH_3)-groups from *S*-adenosyl-Met (SAM) and the reducing power from oxidation of four thiols, from either Trx1 or Grx1 [58]. Arsenic-related elements, including tellurium, strontium, and selenium, are not substrates for AS3MT [68], suggesting the enzyme evolved specifically for detoxifying arsenic. In AS3MT-null mice, arsenic excretion is decreased while tissue arsenic levels are increased, indicating that methylation increases the rate of arsenic excretion [66,69]. However, these mice still have residual levels of methylarsenicals, indicating other processes also methylate As^{III}, but to a drastically lower extent.

Methyltransferases including AS3MT use SAM as the CH_3-donor and generate *S*-adenosyl-Hcy (SAH) as a byproduct, which is subsequently hydrolyzed to release Hcy [70]. Methyltransferase-dependent conversion of SAM to SAH is a vital step in the methionine (Met)-cycle (Figure 2). New CH_3-groups enter the Met-cycle either through acquisition of Met from extracellular sources or from 5-methylenetetrahydrofolate, which in turn obtains the CH_3-group intracellularly from glycine, betaine, or choline [71–73]. In addition to supplying CH_3-groups for methyltransferase reactions, the Met-cycle supplies Hcy to the transsulfuration pathway, which extracts the sulfur (S) from Hcy and uses it to produce either the signaling molecule H_2S or Cys [74,75]. Cys is used for synthesis of protein, GSH, CoA, and other S-metabolites [76]. The combined activities of the Met-cycle, transsulfuration, and GSH biosynthesis can also independently support homeostatic disulfide reducing activities when both TrxR1 and Gsr are disrupted [77,78], as might happen during arsenic exposure (Section 4.5).

4.5. Arsenic interactions with disulfide reductase systems

The affinity of As^{III} for reactive thiols impacts the disulfide reductase systems. Although As^{III} binds GSH with 1:3 stoichiometry, even acute As^{III} exposure doses are likely too low to substantially deplete GSH [19,66,69,79]. As^{III}-bound GSH is only lost when the glutathionylated As^{III} is directly exported [44]; when $As^{III}(GS)_3$ is instead methylated by AS3MT, the GSH is conserved (Section 4.4) [58]. Thus, interactions between GSH and As^{III}, *per se*, do not likely impact redox homeostasis. Nonetheless, binding of As^{III} to GSH might create a "reactivity threshold" that, by preventing low-reactivity thiols from displacing GSH and binding

Figure 2



Cellular redox metabolism in normal and arsenic-exposed cells. In normal cells (top), redox homeostasis is supported by TrxR1 and Gsr, which use NADPH to maintain reduced pools of Trx1 or GSH, respectively. GSH turnover is resupplied by *de novo* GSH synthesis, which is supported in part by Met *via* the intermediates SAM, SAH, and Hcy. As^{III} enters cells (bottom) by various transporters (green, see text) and is glutathionylated to $As(GS)_3$. $As(GS)_3$ is a substrate for AS3MT, the product being methylated arsenicals (MA). $As(GS)_3$ and MAs are exported by MDR proteins (blue). However $As(GS)_3$ also disruptively interacts with the nucleophilic Cys or Sec residues in the active sites of TrxR1, Gsr, and other redox-active enzymes. Genetic knockout models show that co-disruption of TrxR1 and Gsr is compensated by increased Met-fueled flux of sulfur through SAM, SAH, and Hcy to support increased *de novo* synthesis of GSH. The reducing power from GSH is then, in part, cross-trafficked to Trx1, and in combination this supports essential disulfide reductase pathways. Increased flux through this pathway collaterally increases the availability of SAM, the methyl-donor for AS3MT, and thereby further supports arsenic detoxification.

As^{III}, could also effectively focus As^{III}-binding on the highly reactive thiols of critical enzymes, including those within disulfide reductase systems.

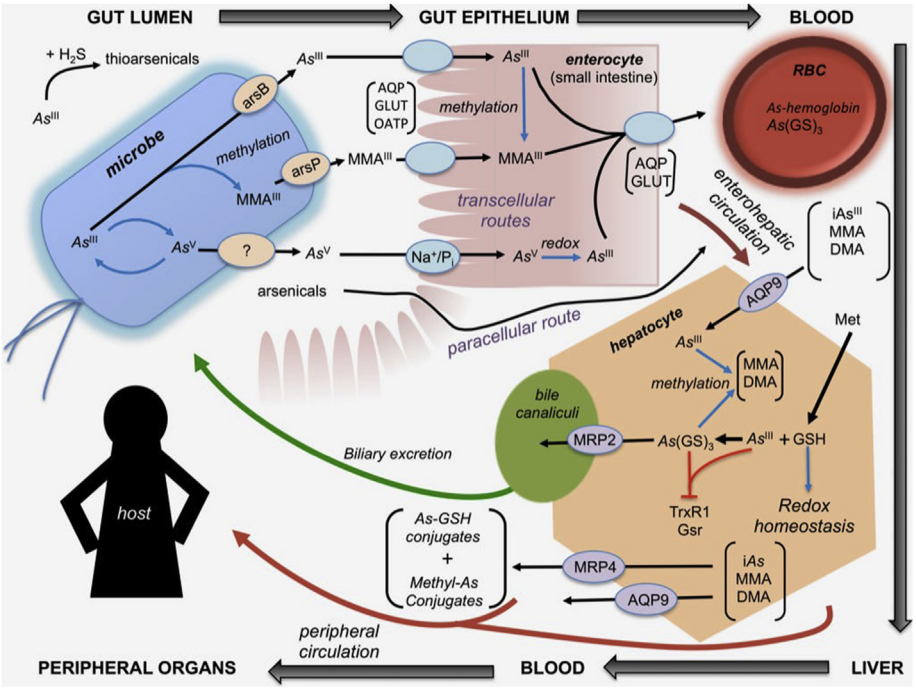
Two high-affinity targets of As^{III} are TrxR1 [11] and Gsr [63], each of which are homologous flavoenzymes [80]. With TrxR1, As^{III} is known to inhibit activity and lead to oxidation of the TrxR1/Trx1 system [11,19]; it is expected that As^{III} will inhibit Gsr by similarly targeting its active site. Critically, these are also the only two enzymes that can use nicotinamide adenine dinucleotide phosphate (NADPH) to reduce cytosolic disulfides [81], making them key for basal cell homeostasis. Indeed, co-disruption of these two enzymes in bacterial, archaeal, or eukaryotic microbes is lethal [82]. By contrast, mammalian cells sustain redox homeostasis in the combined absence of both TrxR1 and Gsr by using an alternative NADPH-independent pathway to generate reduced GSH [77,78,81,83]. The NADPH-independent pathway uses consumption of Met to support *de novo* GSH synthesis, which provides sufficient disulfide reducing power for homeostasis [77]. The ability of mammalian cells to survive disruption of both TrxR1 and Gsr could be a critical survival attribute during arsenic exposure (Figure 2).

Interestingly, the NADPH-independent pathway might also bolster activity of AS3MT by increasing SAM availability [77]. Although both AS3MT and the NADPH-independent disulfide reduction pathway depend critically on GSH, AS3MT recovers the GSH from $As^{III}(GS)_3$ [14], thereby conserving this resource.

4.6. Arsenic transport out of cells

Similar to import, routes of cellular arsenic export depend on the arsenic species present, its concentration, and cell type. In enterocytes, candidate efflux proteins include AQPs, GLUTs, and the multidrug resistance proteins (MRP, or ATP-binding cassette subfamily C proteins, ABCC) 1, 2, and 4 [44]. A similar arsenical exporter profile exists in the liver but also includes the OATPs [44,84]. The only characterized transporter for biliary excretion of arsenic is (ABCC2/MRP2), whose substrates include $As(GS)_3$, seleno-bis-(*S*-glutathionyl) arsinium anion $[(GS)_2AsSe)]^-$, and $MMA(GS)_2$ [44,85,86]. Biliary excretion of arsenic likely is not an effective elimination pathway, as it allows enterohepatic recycling of the arsenic. Most GSH-conjugates dissociate in the alkaline pH of bile [87] and potentially increase the opportunity for gut microbes to produce more toxic thiolated arsenicals. Bu et al. showed that enterohepatic recycling underpinned production of thioarsenicals in rats [88]. MRP4 is likely responsible for efflux of arsenic across the basolateral

Figure 3



Integrated activities of the microbiome and host on arsenic bioavailability. In gut microbes, *iAs* can be reduced, methylated, or thiolated; followed by export. Arsenic species then enter the host through gut epithelium either transcellularly or paracellularly. Within enterocytes, *iAs* can be reduced or methylated, followed by efflux into portal circulation where it can be taken up by red blood cells and GSH-conjugated. Arsenic enters into hepatocytes *via* AQP9. Inside hepatocytes, methylation and glutathionylation can occur. *iAs*, methylated arsenic species, and *As*-GSH conjugates inhibit TrxR and Gsr enzymes in hepatocytes and presumably in all arsenic exposed cells. *As*-GSH conjugates are exported into bile by MRP2, where they recycle through the gut and can be re-assimilated, or into peripheral circulation by MRP4, after which they transit to all peripheral tissues and are eventually excreted in the urine.

membrane of hepatocytes for entry into peripheral circulation [84,89].

5. Conclusions and perspectives

The gut microbiome and enterocytes interact first with ingested arsenic. Each participates in pre-absorptive metabolic processes. Redox metabolism of arsenic in both host and microbial cells results in production of arsenic-metabolites with differing toxicities. The overall health of the host following exposure is likely dependent on exposure, gut metabolism, bioavailability, and the redox metabolism of arsenic inside hepatocytes and peripheral organs (Figure 3). Although most arsenic is eventually excreted in urine, microbiome sequestration can diminish systemic exposure to arsenicals through fecal elimination. The composition of gut microbiota could influence inter-individual susceptibility to arsenic. Thus, probiotics containing bacteria having strategically chosen intrinsic or genetically engineered arsenic-metabolism capacities might be valuable for use in populations that cannot avoid arsenic in drinking water, and might also have value for treating acute exposures.

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Conflicts of interest statement

The authors declare they have no conflicts of interest.

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