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Bioreporters and biosensors for arsenic detection. Biotechnological solutions for a world-wide pollution problem

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A wide variety of whole cell bioreporter and biosensor assays for arsenic detection has been developed over the past decade. The assays permit flexible detection instrumentation while maintaining excellent method of detection limits in the environmentally relevant range of 10–50 μg arsenite per L and below. New emerging trends focus on genetic rewiring of reporter cells and/or integration into microdevices for more optimal detection. A number of case studies have shown realistic field applicability of bioreporter assays.

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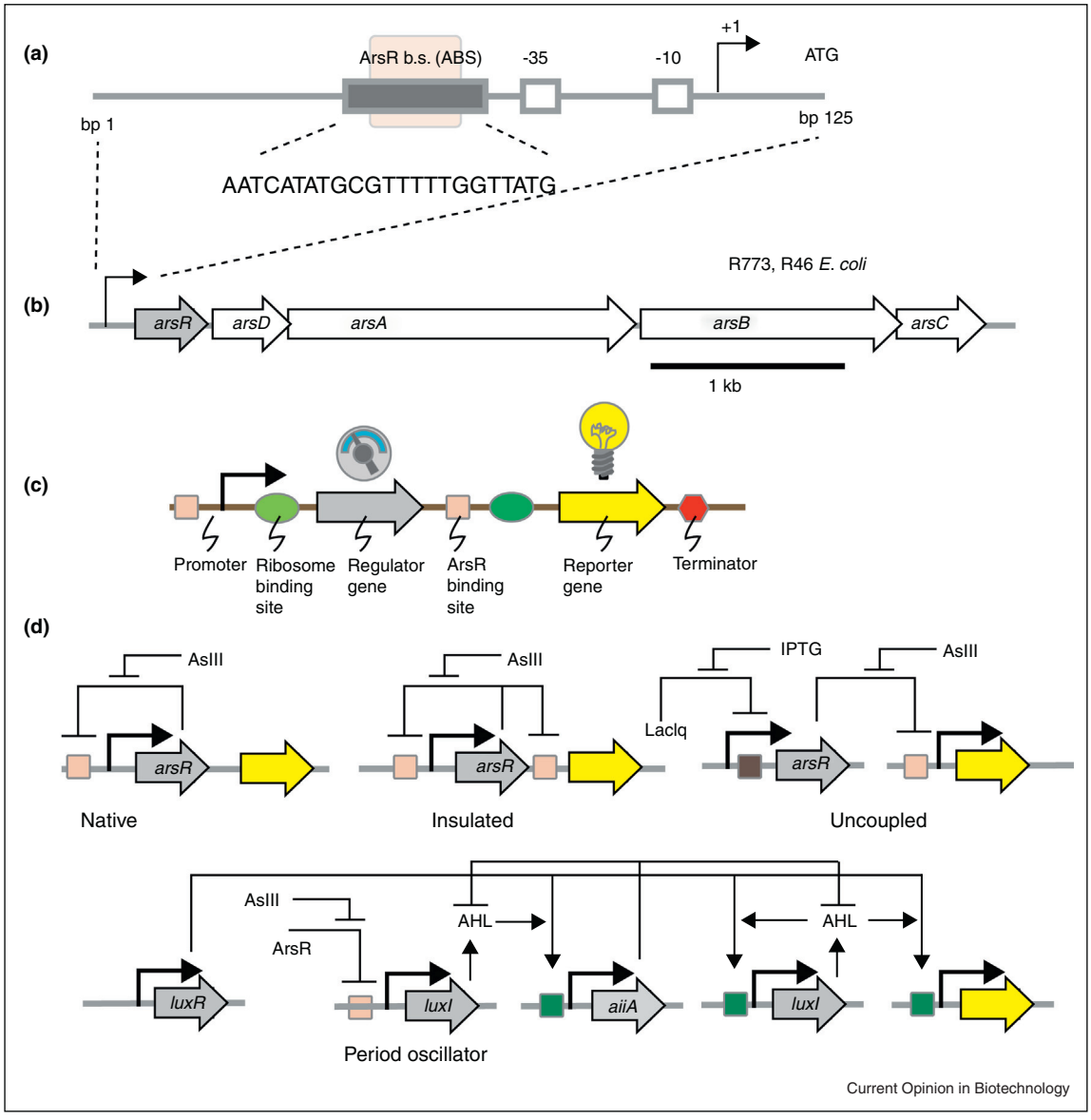
Introduction

Arsenic is an abundant but tightly bound element in the Earth's crust, which, however, under particular hydrogeological conditions and/or as a result of human activities can lead to serious and widespread contamination [1,2,3••]. Arsenic has a complex chemistry and can occur in different inorganic forms, such as trivalent AsIII in As_2O_3, AsO_2^-, AsO_3^{3-} (arsenite) or AsH_3 (arsine), or as five-valent AsV in AsO_4^{3-} (arsenate). In addition arsenic can be bound in organic form, such as in arsenobetaine, trimethylarsine or arsenosugars, often as a result of enzymatic processes [4]. Arsenicals are generally considered as extremely toxic [5], and the provisionally tolerable weekly intake for inorganic arsenic is set at 15 μg/kg of body weight [6] whereas the current WHO recommended drinking water limit is 10 μg/L [1]. The drinking water limit for inorganic arsenic is by far surpassed in potable water sources in large geographical areas such as the deltas of the Ganges, Brahmaputra, and Meghna (India, Bangladesh), Mekong (Loas, Cambodja) or Red River (Vietnam) [3••]. Unfortunately, many of the most affected zones with arsenic groundwater contamination are also those where people have the least access to centralized drinking water systems, and where the logistics of measuring arsenic levels in potable water sources is cumbersome [7]. In addition, accurate quantification of arsenicals requires sophisticated chemical analytics and chemical field-tests are not satisfactory for a variety of reasons [7–9]. Consequently, many research groups have proposed alternative methods for assaying arsenic based on biological systems (e.g. bioreporters and biosensors). The purpose of this current opinion article is thus to review the most recent developments in bioreporters and biosensors for arsenic detection. We will briefly recapitulate the principal concepts of the biological detection of arsenic, then will focus on recent genetic engineering efforts, synthetic biology designs and micro-engineering strategies to incorporate and improve bioreporter assays, and finally will discuss the merits of biological assays under realistic field conditions.

Biological detection of arsenic

Arsenic is not only toxic to higher organisms but also to prokaryotes, and most prokaryotes have evolved exquisite resistance systems against arsenic [10]. Probably the best characterized resistance system to arsenic (and simultaneously to antimonite) was discovered on the plasmid R773 in *Escherichia coli* [11], and is encoded by the *arsRDABC* operon (Figure 1). Expression of the operon is controlled by the ArsR protein, which in absence of arsenic binds as a dimer to an operator DNA called ArsR-binding-site (ABS, Figure 1A) slightly upstream of the −10/−35 promoter [12]. Binding of ArsR to the ABS prevents transcription, but in the presence of arsenic the affinity of the dimer for the ABS decreases, allowing transcription to occur [12]. ArsD is a chaperone protein, which may help to bind and present arsenite to the ArsA ATP-ase part of a specific arsenite efflux pump (ArsAB) [13–15]. Cells can further reduce arsenate to arsenite by means of a reductase (ArsC), which can then again be removed from the cell through the ArsAB efflux pump [15]. Different varieties of the *arsRDABC* operon are found, some of which lack an *arsD* and *arsA* homologue [16], or which carry an additional gene *arsH*, an NADPH-dependent FMN reductase that might prevent re-oxidation of arsenite to arsenate [17]. Arsenic-resistance

Figure 1



Genes and regulatory elements in the design of arsenic bioreporters. **(A)** Detail of the *ars* promoter region of the *arsRDABC* operon of plasmid R773 **(B)**. **(C)** DNA parts representation of a typical *arsR*–P_{ars} based gene circuitry. **(D)** Circuitry functioning of the native ArsR repressor loop, the insulated version with the extra ArsR binding site [24], the uncoupled loop [37], and the period oscillator [38••]. For explanation, see main text. Thick arrows: promoters; thin arrows: activation; flat lines: repression. AHL, acylhomoserine lactone.

operons are also found in anaerobic arsenate-respiring bacteria, which, in addition to an *arsDABC* resistance operon carry the genes for arsenate respiration (e.g. *arrA* and *arrB*) [18]. Recent data suggest that the genes for arsenate respiration are also under control of an arsenite-dependent ArsR-repressable system, the gene for which is located outside the resistance operon [18].

The discovery of the arsenic-binding *trans*-acting ArsR repressor was the basis to propose the genetic engineering of a bacterium (in the following: a 'bioreporter') that would be capable of producing a specific non-cognate reporter protein in response to contact with arsenite [19–22]. The genetic circuit that permits such arsenite-dependent reporter protein formation typically consists of the DNA fragment encompassing the ABS and the *arsR*-promoter, the *arsR* gene and the gene for the reporter protein (Figure 1). Such circuit was constructed several times independently with some small differences in, for example, host background or source of *ars* and reporter genes but with in essence similar functionality [20–24,25•]. Arsenite (or antimonite) entering the reporter cell is thus detected because