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Genetically encoded fluorescent sensors for measuring transition and heavy metals in biological systems

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Great progress has been made in expanding the repertoire of genetically encoded fluorescent sensors for monitoring intracellular transition metals (TMs). This powerful toolkit permits dynamic and non-invasive detection of TMs with high spatial-temporal resolution, which enables us to better understand the roles of TM homeostasis in both physiological and pathological settings. Here we summarize the recent development of genetically encoded fluorescent sensors for intracellular detection of TMs such as zinc and copper, as well as heavy metals including lead, cadmium, mercury, and arsenic.

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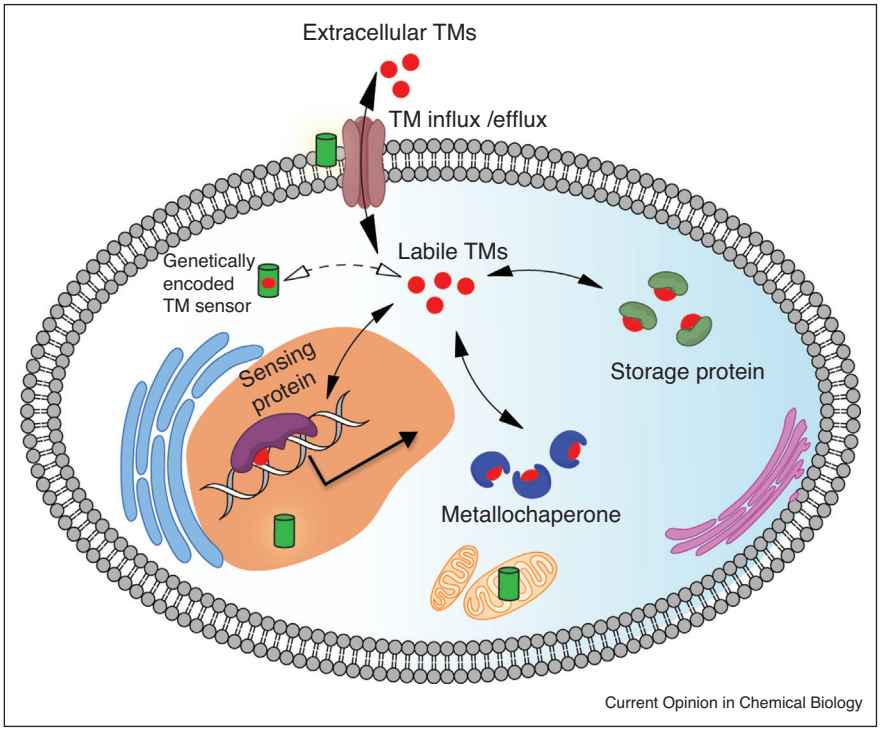
Introduction

Transition metals (TMs) play indispensable roles in numerous biological processes including respiration, gene transcription, enzymatic catalysis, and cell signaling [1•,2], whereas excessive or inappropriate accumulation of these metals will become toxic [1•,2]. The concentrations of most TMs are thus tightly controlled within narrow windows that are fine-tuned via sophisticated machinery inside cells [3]. The TMs contents within a cell comprises a static pool that is tightly bound to proteins and/or ligands as well as a labile pool that is consist of free or/and weakly associated metal ions. These two metal pools are dynamically regulated via metal import and export systems, metallo-sensory proteins, storage proteins, enzymes, chaperones, among others (Figure 1) [3–5]. Recent studies revealed that such TM homeostasis plays crucial roles in host–pathogen interactions [6] and its dysregulation can result in a number of human diseases [1•]. Heavy metals are highly toxic and four of them (arsenic, cadmium, lead, mercury) are listed in the top ten chemicals of major public health concern by the World Health Organization. Heavy metals can induce multiple organ damage on brain, liver, kidney, lungs, as well as on blood composition [7•]. Long-term exposure to heavy metals can also lead to muscular, neurological degenerative processes and cancer [7•]. Despite the fact that heavy metals are highly hazardous, the detailed mechanisms of their overall cellular toxicity have not been fully elucidated [8]. Monitoring intracellular metal ion homeostasis is highly desirable for better understanding their physiological and pathophysiological roles. Unlike traditional detection methods such as flame AAS and ICP-MS, fluorescent probes are powerful tools for direct visualization of these metal ions inside living cells with high spatial-temporal resolution. Both synthetic probes and genetically encoded biosensors have been extensively developed in recent years, and each approach has its unique advantages [9,10••]. For example, unlike extraneous synthetic dyes, genetically encoded sensors, most of which are fluorescent protein (FP)-based biosensors, have lower perturbation to intracellular metal homeostasis. Notably, genetically encoded sensors can be selectively localized to subcellular organelles by fusing with specific signal sequences, which overcomes the issues of non-specific leakage and cellular diffusion of synthetic probes. Important progress has been made in detecting and visualizing intracellular metals using FPs. In this review, we focus on the recent development of genetically encoded fluorescent sensors for measuring the labile pool of TMs and heavy metals in living systems. Remaining challenges and future perspectives are discussed at the conclusion.

Category and design considerations for FP-based biosensors

Most of genetically encoded TM sensors are developed by utilizing metal sensing/binding motif in combination with one or more FPs. Generally speaking, the sensing motif for TM detection in living systems needs to meet several desired criteria such as high selectivity for metals of interest, and appropriate binding affinity that matches the intracellular availability of the labile metal pool. The toolkit of genetically encoded sensors can be divided into two categories based on the FPs being used:

Figure 1



A brief model for TM homeostasis and the intracellular expression of genetically encoded TM sensors. TMs are maintained within an optimal bioavailable concentration that is regulated by a network of machineries in controlling metal uptake/efflux, metal sensing, trafficking and storage processes. Genetically encoded TM sensors can be applied to monitor intracellular labile TMs.

FRET/BRET-based sensors, and single FP-based sensors (Figure 2). A FRET-based metal sensor consists of a pair of FPs (FRET-pair) that is linked by a metal sensing domain/peptide with high affinity and specificity toward the target metal ions. The conformational changes of the sandwiched metal-sensing motif upon metal binding or recognition affects the FRET efficiency that can be reflected by the change of emission ratio of the FRET-pair (Figure 2a–d). A notable advantage of such FRET based sensors is that their fluorescence is ratiometric, allowing the elimination of artifacts in protein concentration, sample thickness, and movement. The choice of the FRET pair is also rapidly growing, ranging from the traditional ECFP/EYFP FRET pairs [11] to the further optimized CFP/YFP derivatives [12], and to the bright photostable pairs such as mClover/mRuby [13,14]. However, due to the limited structural conformational changes in response to metal binding, the signal change of FRET is often moderate. To achieve better emission ratio change, much work has been focused on adjusting the flexible linker length [15,16] or implementing self-associating mutations at the dimerization interface of FPs [17••]. For example, Merkx and coworkers developed self-associating FP variants by introducing S208F and V224L on both ECFP and EYFP in the CALWY (CFP-Atox1-Linker-WD4-YFP) Zn(II) sensor, which resulted in a 6-fold enhancement of dynamic range [17••,18]. This strategy has also been expanded to the mOrange/mCherry pair to generate improved red-shift FRET sensor for Zn(II) detection [19].

Single-FP-based metal sensors undergo fluorescent intensity change via variation in local environment of FP chromophore upon binding or interacting with the metal ions. This strategy can be realized by inserting an intrinsic metal sensing domain into the FP or introducing mutations on FPs to directly bind metals (Figure 2e–g). In particular, circularly permutated FPs (cpFPs) have been developed that can undergo fluorescence intensity change upon interaction with metal ions [20,21]. Compared with the FRET-based sensors which are ratiometric, single-FP-based intensiometric metal sensors have broader dynamic range, and narrower excitation/emission wavelengths. These properties make single-FP-based intensiometric sensors a better choice when higher signal/noise ratio is required, or if there is simultaneous application of multicolor fluorescent sensors. Nevertheless, the ratiometric FRET-based sensors are preferred in a quantitative measurement (e.g. measuring metal concentrations in subcellular compartments), as intensiometric sensors may be affected by the variation in sensor concentration and/or fluctuation of optical path length.