Searching for harmony in transition-metal signaling

Christopher J Chang

The recent emergence of signaling roles for transition metals presages a broader contribution of these elements beyond their traditional functions as metabolic cofactors. New chemical approaches to identify the sources, targets and physiologies of transition-metal signaling can help expand understanding of the periodic table in a biological context.

The periodic table represents a chemical blueprint for organizing the elements of life. By analogy to an orchestra, each element can be thought of as an instrument with a unique sound and personality. In this context, chemical biologists not only can listen passively, uncovering and appreciating the music that nature plays, but also can actively compose and conduct new collections of these instruments to create new music that is greater than the sum of its parts. Perhaps nowhere is this concept better illustrated than in the chemical biology of metals, which offers a molecular diversity that far surpasses that of pure organic systems, owing to a greater inherent flexibility in coordination number and oxidation state.

Indeed, metals pervade all aspects of the central dogma because they are essential for the function of all DNA and RNA molecules, and one-third to one-half of all proteins, in addition to contributing to epigenetic modifications[1]. Additionally, unique properties of these elements, such as spin or radioactivity, can be harnessed to create new diagnostics and therapeutics as a part of the frontier area of metals in medicine.

Despite this wealth of untapped chemical breadth, metals have been traditionally divided into one of two categories: dynamic signals or static cofactors (**Fig. 1a**,**b**). Redox-inactive alkali and alkaline earth metals, such as sodium, potassium and calcium, along with divalent zinc in certain cases, have been broadly recognized as signals through fast, orchestrated movement and exchange of metal ion pools[1,2] (**Fig. 1a**). Redox-active transition metals such as copper and iron, owing to their potential to trigger oxidative stress and damage via Fenton chemistry when unregulated, have been studied largely as static cofactors that must be buried and protected within protein active sites for structural and/or catalytic functions[1] (**Fig. 1b**). In a broader sense, elements co-opted for cell signaling involve mainly ionic interactions, as in the case of closed-shell sodium, potassium or calcium, or covalent modifications, as in the case of phosphorus (phosphorylation), carbon (glycosylation or lipidation) and oxygen (oxidation)[2]. Relatively less attention has been paid to the capacity of transition metals, which can participate in both ionic and covalent bonding, for cell signaling (**Fig. 1c**).

This restrictive notion that metals are either signals or cofactors ignores a fundamental property of metals: that they can be neither created nor destroyed under most ambient physiological conditions. In other words, metal homeostasis must be maintained by coordinated uptake, trafficking and efflux pathways that place the right metal in the right amount at the right place and time in the cell. Indeed, elegant efforts in the cell biology of metals continue to identify principles of metal regulation across both the periodic table and all forms of life. As such, the variable thermodynamics and kinetics of metal exchange mean that there is a continuum that blurs the lines between metabolism and signaling, placing metals in a unique chemical and biological space.

In this Commentary, I put forth select examples to support this emerging paradigm of transition-metal signaling, defined as transition metal–biomolecule interactions that govern and coordinate biological activities in response to stimuli. In this context, I propose a simplified generalized workflow of source, target and physiology (STP) to study these interactions (**Fig. 2**), highlighting where chemical tools and tactics can enable new biological discoveries, which in turn can further inform the design of new chemical technologies. In particular, I highlight the use of imaging probes to identify sources, inhibitors to identify targets, and chelators and ionophores to disentangle physiology in a diverse range of biological models. This focus on dynamic processes of transition metals in native systems—going beyond the canonical molecular study of individual metal active sites, such as those of metalloproteins and metal–nucleic acid complexes—broadens the conventional scope of bioinorganic chemistry and metals in biology. Finally, I propose further opportunities to engage in studies of the periodic table of life.

Zinc reproductive biology

As a starting point, I highlight an exciting example in which synthetic and physical imaging probes have identified that zinc-based transition-metal signaling begins at the initial stages of life. Zinc is an essential cofactor in all classes of enzymes and is a major regulator of gene expression via tightly bound zinc-finger domains[1]. Since the 1950s, labile pools of zinc, defined as loosely bound stores that can be attenuated by acute chelation, have been visualized by histochemical and fluorescent stains in human cells and tissues, including the brain, pancreas, prostate, testes and intestines[3]. Despite these early observations, the physiological functions of such pools remain insufficiently understood.

Recent work has exploited cutting-edge synthetic and physical imaging tools to identify, and in some cases quantify, vesicular zinc sources to study zinc physiology in mammalian oocytes. Using synchrotron-based X-ray fluorescence microscopy (XFM), which provides direct measurements of total metal pools in fixed samples with spatial resolution, a zinc-dependent checkpoint in oocytes during meiosis has been identified, in which zinc accumulation during oocyte maturation is required to complete the process[4]. Further imaging work combining

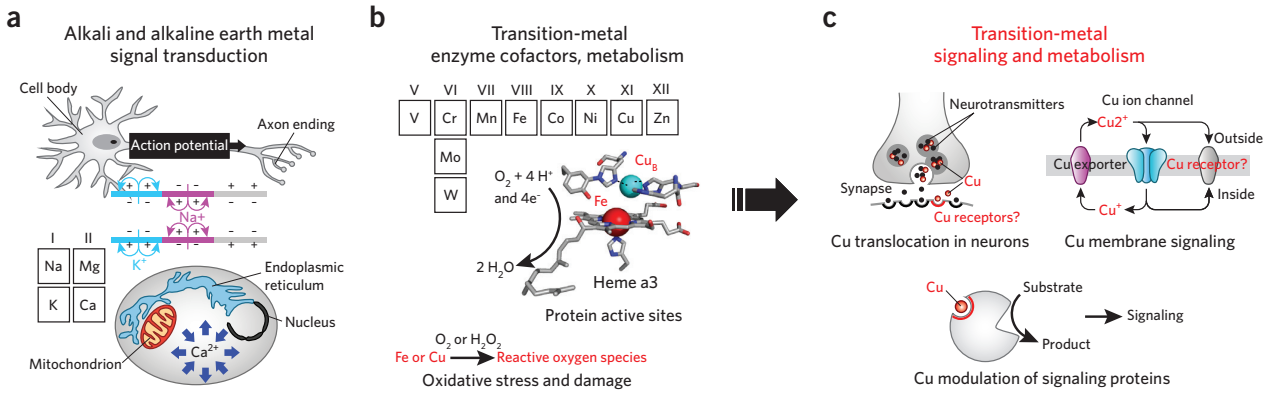

Figure 1 | Transition-metal signaling. (**a**) Cell signaling is largely based on dynamic movements of redox-inactive metals such as sodium, potassium and calcium in kinetically labile forms that reversibly bind cellular targets. (**b**) Transition metals, particularly redox-active metals such as copper and iron, have been traditionally thought to be more kinetically inert cofactors that are tightly bound and buried within proteins and nucleic acids, because of their potential toxicity to cause oxidative stress and free-radical damage. (**c**) Examples of transition-metal signaling with copper include activity-dependent copper translocation in neurons and other cell types, copper ion channels that mediate signaling across membranes, and reversible copper binding to intracellular signaling proteins such as kinases to modulate their activity.

XFM and a synthetic fluorescent zinc probe has revealed zinc release upon fertilization of the egg[5]. A comprehensive and quantitative study combining a suite of complementary imaging techniques—including a new chemical fluorescent probe for live-cell zinc imaging, XFM, three-dimensional elemental tomography, scanning transmission electron microscopy (STEM) and energy-dispersive spectroscopy (EDS) with a modified histochemical staining protocol—has established that these 'zinc sparks', which are essential for the egg-to-embryo transition, originate from a system of thousands of vesicles, each of which contains approximately 10^6 zinc atoms[6]. This work sets the stage for illuminating the targets of these vesicular sources of zinc in egg and sperm physiology.

Metal cancer-signaling pathways

Connections between transition-metal metabolism and signaling have emerged recently in the cancer field. In this context, the altered metabolism of cancer cells via elevations in anaerobic glycolysis (for example, the Warburg effect) with dampened mitochondrial function has continued to be of central importance in these diseases[7]. Mitochondria, of course, require redox-active iron and copper as metabolic cofactors for enzymes that catalyze electron-transport and oxygen-reduction reactions in aerobic respiration.

However, two emerging stories now connect copper and iron signaling to two essential but opposite ends of the cancer spectrum: cell growth and proliferation, and cell death. On the side of oncogenic cell growth and proliferation, copper has been identified as a kinase cofactor in fly and mouse systems, and copper influx through Ctr1 enhances MEK1 phosphorylation of ERK1/2 through a copper-MEK1 interaction[8]. Characterization of this target of transition-metal signaling with the aid of genetic and pharmacological manipulations has set the stage for the physiological finding that copper is required for oncogenic MEK-dependent BRAF signaling and tumorigenesis in mouse and human cell settings[9]. Because mutation of the BRAF kinase induces an active oncogenic state in a large fraction of melanomas and leukemias, as well as thyroid and other cancers, this work suggests that copper-chelation therapy could be repurposed to treat these types of cancers and related diseases that share this genetic basis and/or signaling pathway.

A second elegant example of transition-metal signaling in cancer has identified a new form of physiology via iron-dependent cell death, termed ferroptosis[10,11]. This cell-death pathway was uncovered in the course of studying the mechanism of action of erastin, a small molecule that selectively kills cells expressing oncogenic mutants of RAS. Further mechanistic studies with targeted metabolic profiling have identified that depletion of glutathione causes inactivation of glutathione peroxidase 4 (GPX4), revealing that the latter target is an essential regulator of ferroptotic cell death[12]. The discovery of a specific new cell-death pathway relying on the synergy between metal and redox activity illustrates that transition metals can have distinct chemical roles inaccessible by ionic alkali and alkaline earth and covalent carbon, phosphorus or oxygen modifications. In addition, these examples highlight the power of small-molecule inhibitors as chemical tools to help identify targets for physiological studies. Sources of copper and iron that elicit these physiological responses remain unknown.

Probing metalloneurochemistry

The brain is the center of everything we see, hear, touch, taste, smell, feel and learn, and unlocking the mysteries of how a combination of chemical elements can form the center of consciousness itself is a perennial quest. In this context, it is fascinating that brain tissue contains among the highest levels of metals in the body[13,14]. Metalloneurochemistry involves the study of metal ion function in the brain and nervous system at a molecular level[15], and recent advances have shown how zinc and copper are essential to basic neural function.

Because fluorescent sensors for zinc have been extensively reviewed[13,16], I will focus on another set of chemical tools—chelators—which have helped to disentangle the physiological actions of zinc by rapid and selective depletion of endogenous labile zinc stores[17,18]. This chemical biology approach offers a complementary advantage to highly specific genetic manipulations, owing to the fast and reversible action of small-molecule pharmacophores, which minimizes potential compensatory transcription and translation mechanisms from genetic overexpression, knockdown or knockout.

In particular, the development of a kinetically rapid and selective zinc chelator has enabled the identification of two key principles of neural zinc physiology through vesicular zinc sources. The first principle

is the coupled promotion of presynaptic and inhibition of postsynaptic long-term potentiation in the hippocampal mossy fiber synapse, a region in the brain that is involved in long-term memory and accumulates an unusually high amount of labile zinc in vesicular sources. A more recent study has applied this chelator to show that both synaptic zinc and tonic zinc can modulate extrasynaptic NMDA-receptor targets[19].

On the redox-metal side, work from our laboratory has provided evidence for labile copper's acting as an endogenous regulator of neural function, identifying sources of dynamic copper that act on copper-dependent targets to control brain physiology. Using a combination of a second-generation fluorescent copper sensor and XFM, we have observed that neural activity triggers movements of copper from somatic-cell body sources to dendritic processes and established that these copper fluxes are calcium dependent[20]. More recently, using next-generation fluorescent copper indicators with improved hydrophilicity along with matched control dyes that lack copper sensitivity, we have identified endogenous sources of labile copper in the developing retina by one- and two-photon microscopy and showed that they modulate spontaneous activity, a fundamental physiological property of neural circuits[21]. Both acute and reversible pharmacological treatment with the copper chelator bathocuproine disulphonate (BCS) as well as genetic knockout of the copper importer Ctr1 have provided evidence for a general physiological role for copper in neural function. The latter data suggest the notion of a 'copper ion channel' as a target for this physiology.

Using the STP framework

The foregoing examples of transition metal signaling provide a snapshot of the open opportunities in this nascent area and point to new directions for studies of metals in biology from a chemical perspective. In this context, new chemical tools and tactics to help identify any and all parts of this vast biology will be extremely valuable. Indeed, **Figure 2** gives a workflow example in which a base STP framework comprising a source (Golgi), target (Ctr1) and physiology (spontaneous activity) of copper signaling in the brain has been elucidated. This framework was developed over several studies using multiple emerging chemical technologies, including synthetic and physical imaging probes for characterizing metal sources and dynamics, genetic and pharmacological manipulations for identifying metal targets, and metal chelators and sensors for probing metal physiology. Owing to space limitations, this list is not comprehensive but is meant to trigger further consideration of how understanding the chemical biology of metals can be advanced.

Where are the metal sources? Signaling is all about location, and because metals cannot be created or destroyed, they are well suited to contribute to physiology through coordinated spatial and temporal changes in their local concentrations and ligation status. Because dynamic changes in both total and labile metal pools can involve movements, redox changes, full or partial ligand exchanges and/or redox and nonredox catalysis, many open questions remain with regard to characterizing metal sources from the single-molecule to organelle to cell to tissue to organismal levels. In this context, small-molecule and macromolecule imaging probes for metal ions and other inorganic species represent just one area of rapid growth and continued need[13,16,22,23]. These tools will be complemented by bulk techniques for direct analysis, particularly ones that are suitable for spatial imaging, for example, XFM and nanoscale secondary-ion MS (nanoSIMS). Improved chelators and ionophores with variable kinetics and thermodynamics, and predictable cellular and tissue localizations, as well as caged probes that afford spatial and temporal control of metal pools, are also critical to the field[24].

An important consideration is that there is no one-size-fits-all technology: owing to the complexity and diversity of biology, the right chemical tool for one application or model may not work as effectively in another. Indeed, it is critical that chemical characterization of any tool be accompanied by direct biological evaluation for each given situation. The combination of monitoring total and labile metal pools, as well as rapid pharmacological depletion or expansion of such pools, will continue to improve the ability to characterize metal sources.

What are the metal targets? Target identification is also a major unmet need because the same diversity of coordination number and geometry that makes metal sites so chemically rich also makes them difficult to predict and find a priori[1], especially given the possibilities of promiscuous binding of multiple metals. Advances in metalloproteomics, including improvements in computational prediction and analysis, as well as increased attention to small-molecule inhibitors and activators of biologically relevant metal-binding sites, represent an open frontier for research at the inorganic chemistry–biology interface. These emerging technologies, along with continued development of genetic tools, for example, clustered regularly interspaced short palindromic repeats (CRISPR) for knockdown and knock-in experiments, will advance the field by expanding the number of recognized and validated metalloprotein, metallo-DNA, metallo-RNA, metalloglycan, metallolipid and related interactions in living systems.

How do metals contribute to physiology? Understanding how metals influence the physiology of all forms of life and their interactions with the environment is the largest area of growth for the metals field. In addition to the aforementioned examples in reproductive biology, cancer and neuroscience, other areas such as immunology (including environmental microbiology, the microbiome, innate and adaptive immune systems, and more complex systems such as neural-immune

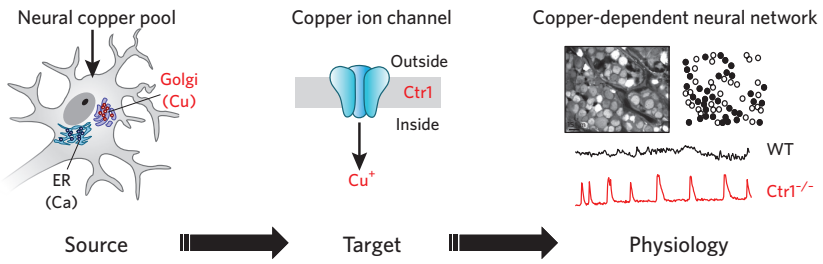

Figure 2 | A source-target-physiology (STP) workflow for studies of transition-metal signaling, as exemplified by studies of copper physiology in neural systems. Chemical biology can play a key part in this research, as evidenced by the unmet needs for new technologies for visualizing and manipulating metal pools as well as identifying new metal-binding sites in biological systems. Imaging by XFM and a chemical fluorescent probe identifies labile copper pools in neurons (source), which upon either depolarization or spontaneous activity can act via Ctr1 (target). Acute, reversible pharmacological depletion of basal labile copper pools by chelation or targeted genetic knockdown of the Ctr1 target establish the requirement for copper for normal spontaneous activity, which is a fundamental property of all neural circuits (physiology), as measured by two-photon calcium imaging. ER, endoplasmic reticulum; WT, wild type.

interactions), plant biology (particularly with regard to agricultural issues such as food consumption), cardiovascular function and disease, stem-cell biology and regenerative medicine, and epigenetics represent just a few of the topics in which contributions of metal physiology are insufficiently understood at a molecular level. A remaining challenge for the field will be to expand the diversity of model systems and of translational research focused on metals. The contribution of metals to physiology remains insufficiently explored even in standard model organisms, much less in microbial systems, leaving much room for collaboration, particularly given recent advances in genetics.

Outlook

A final consideration is that chemistry must be done in dialog with biology and vice versa, to leverage the expertise of both disciplines. Recent examples of the effective use of multiple approaches combining thorough physical characterization techniques and analysis with interesting biological questions, in a spirit of open collaboration[6,25], will continue to make the periodic table a place where chemistry and biology join together and play in harmony.

Christopher J. Chang is at the Departments of Chemistry and Molecular and Cell Biology, Helen Wills Neuroscience Institute, and Howard Hughes Medical Institute, University of California, Berkeley, Berkeley, California, USA, and the Division of Materials Biology, ShanghaiTech University, Shanghai, China.
e-mail: chrischang@berkeley.edu

References

1. Lippard, S.J. & Berg, J.M. *Principles of Bioinorganic Chemistry* (University Science Books, Mill Valley, California, USA, 1994).
2. Alberts, B. *et al. Molecular Biology of the Cell* 5th edn. (Garland Science, Taylor & Francis Group, New York, 2008).
3. Maske, H. *Naturwissenschaften* **42**, 424 (1955).
4. Kim, A.M., Vogt, S., O'Halloran, T.V. & Woodruff, T.K. *Nat. Chem. Biol.* **6**, 674–681 (2010).
5. Kim, A.M. *et al. ACS Chem. Biol.* **6**, 716–723 (2011).
6. Que, E.L. *et al. Nat. Chem.* **7**, 130–139 (2014).
7. Vander Heiden, M.G., Cantley, L.C. & Thompson, C.B. *Science* **324**, 1029–1033 (2009).
8. Turski, M.L. *et al. Mol. Cell. Biol.* **32**, 1284–1295 (2012).
9. Brady, D.C. *et al. Nature* **509**, 492–496 (2014).
10. Dixon, S.J. *et al. Cell* **149**, 1060–1072 (2012).
11. Dixon, S.J. & Stockwell, B.R. *Nat. Chem. Biol.* **10**, 9–17 (2014).
12. Yang, W.S. *et al. Cell* **156**, 317–331 (2014).
13. Que, E.L., Domaille, D.W. & Chang, C.J. *Chem. Rev.* **108**, 1517–1549 (2008).
14. Bush, A.I. *Curr. Opin. Chem. Biol.* **4**, 184–191 (2000).
15. Burdette, S.C. & Lippard, S.J. *Proc. Natl. Acad. Sci. USA* **100**, 3605–3610 (2003).
16. Carter, K.P., Young, A.M. & Palmer, A.E. *Chem. Rev.* **114**, 4564–4601 (2014).
17. Kawabata, E. *et al. J. Am. Chem. Soc.* **127**, 818–819 (2005).
18. Pan, E. *et al. Neuron* **71**, 1116–1126 (2011).
19. Anderson, C.T. *et al. Proc. Natl. Acad. Sci. USA* **112**, E2705–E2714 (2015).
20. Dodani, S.C. *et al. Proc. Natl. Acad. Sci. USA* **108**, 5980–5985 (2011).
21. Dodani, S.C. *et al. Proc. Natl. Acad. Sci. USA* **111**, 16280–16285 (2014).
22. Chan, J., Dodani, S.C. & Chang, C.J. *Nat. Chem.* **4**, 973–984 (2012).
23. Aron, A.T., Ramos-Torres, K.M., Cotruvo, J.A. Jr. & Chang, C.J. *Acc. Chem. Res.* **18**, 2434–2442 (2015).
24. Franz, K.J. *Curr. Opin. Chem. Biol.* **17**, 143–149 (2013).
25. Hong-Hermesdorf, A. *et al. Nat. Chem. Biol.* **10**, 1034–1042 (2014).

Acknowledgments

I thank the Howard Hughes Medical Institute as well as the US National Institutes of Health (grant GM 79465) for generous research support of my interests in the study of metals at the chemistry-biology interface. I thank A. Aron for helpful feedback on an earlier draft of this article.

Competing financial interests

The author declares no competing financial interests.