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Opinion

mcmd 8/22/2020 very important article that suggests Zn can help balance metabolism (chemical processes) as well as its chelation by certain mold toxins (as a way of attack).f
Also discusses errors in much bio research based on the media containing fluoride as well as insufficient nutrients.

Zinc, an unexpected integrator of metabolism?

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Summary

Even when they no longer require the presence of iron, cells use zinc as a divalent cation, involved in a large variety of catalytic and regulatory functions. This metal is so important that it appears that ribosomes are instrumental in its ultimate storage. Here, we summarize a detailed analysis which investigates the way the global cell metabolism is integrated by zinc. This integration results from the zinc-dependent way in which the one-carbon metabolism is always coupled to the translation process, not only *via* methionine and *S*-adenosylmethionine, but *via* the complex set-up of the modification of the position 34 of the anticodon of tRNAs.

Much more than an anecdote: we keep behaving as the medieval alchemists who achieved the transmutation of metals. In contrast with physics, biology does not take great care about many details of its experimental implementations. When we grow microbes in 'defined' media – that is media supposed to contain all the ingredients required to support life [see reference recipes in (Miller, 1972; Lessard, 2013)] – many elements found in the cells come from nowhere. This is true even for metabolic engineering. To be sure, historically, biotechnology developed from industrial processes that were still plagued with vitalistic insights. Louis Pasteur changed the paradigm when he refuted the idea of spontaneous generation and decided to study the 'diseases' of beer and wine. However, while Pasteur took great care in the way he sterilized and kept the media he recommended, he did not consider the details of the growth media, except for their standard industrial set-up. Alas, we know that the way we grow cells triggers considerable variability and inconsistencies in our observations [see, e.g. (Sridhar and Steele-Mortimer, 2016)]. Here is a striking illustration that tells us that we must change the way we go. For decades, biochemists used supplementation of assay media with the fluoride ion as a way to explore energy metabolism (Elsbach and Schwartz, 1959; Manno and Schachter, 1970), PEP-dependent transport (Cirillo and Razin, 1973; Saier and Feucht, 1980) or the activity of adenylate cyclase *via* interference with guanine nucleotide-binding proteins [G-proteins, (Tao and Lipmann, 1969; de Haën, 1974)]. The role of fluoride long remained 'a frustrating mystery' (Maguire *et al.*, 1975). Having serendipitously found that changing glassware for plasticware abolished the fluoride effect, Sternweis and Gilman reported that the purified G-protein regulation of adenylate cyclase depended on the presence of aluminium traces (Sternweis and Gilman, 1982). Subsequently, Chabre discovered that fluoride extracts aluminium from glass, forming the AlF_4^- anion, isosteric with phosphate, and takes its place (Bigay *et al.*, 1985). AlF_4^- is since then used as an authentic mimic of phosphate (Jin *et al.*, 2017).

Yet, despite cogent examples such as this one, we use costly and time-consuming technology to study living processes still based on their unfolding in uncontrolled environments. It is therefore no surprise that reproducibility/replicability is not a strong marker of 'scientific' publications in the domain of biology (National Academies of Sciences, Engineering, and Medicine; Policy and Global Affairs; Committee on Science, Engineering, Medicine, and Public Policy; Board on Research Data and Information; Division on Engineering and Physical Sciences; Committee on Applied and Theoretical Statistics; Board on Mathematical Sciences and Analytics; Division on Earth and Life Studies; Nuclear and Radiation Studies Board; Division of Behavioral and Social Sciences and Education; Committee on National Statistics; Board on Behavioral, Cognitive, and Sensory Sciences; Committee on Reproducibility and Replicability in Science, 2019). What if one of the overlooked components played a critical role in major life processes? We have now hints that zinc, as the divalent cation Zn^{2+} and not monitored in the vast majority of growth media or biochemical assays, is integrating major metabolic

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features *via* the coordination of translation with one-carbon metabolism [Danchin *et al.* 2020 and Fig. 1].

How is this unfolding? Zinc is a fairly unobtrusive metal in life processes but it is referenced in many articles (almost 150 000 at PubMed in January 2020) despite its still elusive behaviour as a biochemical entity (Krezel and Maret, 2016). The most familiar role of zinc is related to its presence in 'zinc-finger' domains of proteins, in particular regulatory proteins or protein–protein interfaces (Eom *et al.*, 2016). As cases in point, zinc-finger proteins have been used as molecular scissors for gene editing before the advent of the CRISPR-Cas9 technology and they are still used (Broeders *et al.*, 2019). Zinc is a critical cofactor in a great many enzymes – as much as 9% of the entire proteome in eukaryotes and from 5% to 6% in prokaryotes (Andreini *et al.*, 2009). Yet, its singularity has been virtually overlooked [see however studies such as (Bütof *et al.*, 2019)]. Unexpectedly, it was recently observed that the *Aspergillus fumigatus* mycotoxin, gliotoxin, had a function specifically involving zinc (Seo *et al.*, 2019), and, because this metabolite could act as a specific zinc chelator, this opens up approaches to investigate in depth the role of zinc.

Indeed, the main reason for ignoring metal ions in growth media stems from the considerable difficulty we have to carefully control their concentration. Transition metal ions, in particular, lose their $4s^2$ electrons, and the corresponding Me^{2+} ions have more or less the same size, with fairly common hydration properties. Also, transition metals tend to favour binding to the same atoms (oxygen, nitrogen, also sulfur). The consequence is that it is difficult to distinguish between their individual transport systems or binding sites. A rule of thumb tells us that, when catalysis involve redox reactions, the nature of the cation has considerable influence on the outcome of the reaction, while non-redox reactions – typical of reactions involving Zn^{2+} such as hydrolases (Coleman, 1998) – are much less sensitive to the ion (Valasatava *et al.*, 2018). The consequence is that it is next to impossible to use standard chelators such as ethylenediaminetetraacetate (EDTA) to exert a tight control on their relative concentration (Chen *et al.*, 2008). This same obstacle is likely to exist also for the cell, which has to scavenge many metals from its environment. This circumstance created a remarkable selective pressure that resulted in the design of highly specific chelators or storage proteins. However, while the process has been explored for the acquisition of iron – showing competition or cooperation *via* a large variety of siderophores with cells creating progressively stronger siderophore to outcompete those which make weaker ones (Kramer *et al.*, 2019) – comparable studies have seldom been extended to other metals. The widespread interest in competition for iron as a major trigger of microbial virulence (Palmer and Skaar, 2016) made that investigators overlooked much details of how cells would acquire other metal divalent ions. Yet, in the case of zinc, it is well established that the cation is both essential and toxic if too concentrated [see a general discussion, centred on

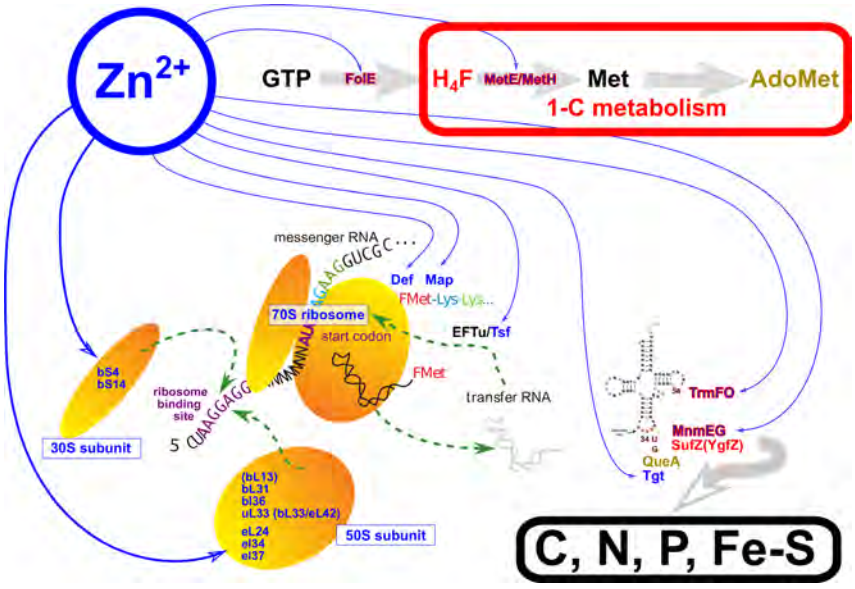

Fig. 1. Zinc as an integrator of metabolism. Zinc-binding proteins are in blue. When they also bind tetrahydrofolate, they are bordered by red. Methylations are not displayed, but the role of S-adenosylmethionine (AdoMet) in the formation of queuine is indicated in brown. The ribosome acts as a Zn^{2+} store. The MnmEG/SufZ complex acts as a coordinator of carbon, nitrogen, phosphate and iron-sulfur metabolism.

plants in (Cabot *et al.*, 2019)]. As a consequence, its availability must be exquisitely regulated by a homeostatic process.

In a surprising turn of things, the intricate coupling between one-carbon metabolism and translation – initially witnessed as the omnipresent requirement for a methionine residue at the start of translated polypeptides – revealed that the connection was established *via* the homeostasis of Zn^{2+} (Danchin *et al.* 2020, and Fig. 1), mediated by the ribosomes as a main Zn^{2+} store (Hensley *et al.*, 2012). To be sure, in the three domains of life, several ribosomal proteins comprise a zinc-binding domain. Furthermore, in Eukarya a number of ribosomal proteins also bind zinc-finger regulators [see, *e.g.* (Dionne *et al.*, 2019)]. This role must now be connected to the observation that in the model bacterium *Bacillus subtilis* a sophisticated regulatory circuit was recruited to optimize Zn^{2+} acquisition and cellular distribution when the ion becomes limiting (Nies, 2019). Miscellaneous studies suggest that this role of zinc is general. Homeostasis is based on zinc-dependent enzymes – in particular enzymes involved in folic acid synthesis – that probe the pool of available Zn^{2+} ions and then amplify this signal to control the activity of Zn^{2+} chaperones, as well as modulation of the zinc content of ribosomes.

what does "three domains" mean?

What is more, many zinc-dependent enzymes that are required for translation – with tRNA modifications as substantiating evidence in all domains of life – are directly dependent on 1-C metabolism, not only *via* methylations but also directly involving tetrahydrofolate. This strongly argues for a functional dependency relating translation, 1-C metabolism and zinc homeostasis. Analysis of the underlying enzyme activities revealed that this possibly happened via management of iron-sulfur clusters (Danchin *et al.* 2020). The key metabolites of this network are formaldehyde, methionine, S-adenosylmethionine (AdoMet) and tetrahydrofolate. Remarkably, a triad of tRNA anticodon modification enzymes, namely MnmE, MnmG and SufZ (YgfZ), are integrated together *via* a role of zinc coupling translation, folic acid metabolism, management of iron-sulfur clusters and zinc availability. The MnmEG complex, previously identified as coupling carbon metabolism with replication (Shippy and Fadl, 2015) as a consequence of tRNA modifications, is further used for phosphate homeostasis by thiolation of the same U34 base (Gupta *et al.*, 2019), making this position ideal to balance carbon, nitrogen, sulfur and phosphate metabolism *via* a Zn^{2+}-mediated translation control of Fe^{2+}-S cluster synthesis, input into polypeptides during translation and maintenance.

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! Zn can help balance some important components of metabolism

Finally, this puzzling role of zinc asked for an investigation of its origins, in particular by revisiting the available scenarios of the origins of life – essentially plagued by creeds, and fairly far from authentic science. If the metabolic origin of biological macromolecules was distributed within large populations of cells that kept exchanging metabolic pathways and primitive genetic set-ups, we favoured the scenario proposed by Charles Kurland where primitive cells made populations of fairly large scavenger organisms (a last ancestral cell ensemble – LACE) in a predator/ prey dialog (see https://www.youtube.com/watch?v=3iD0RiNw9B4 and Danchin *et al.* 2020). In this scenario, H_4F, formate and nucleotides were primitive compounds. They were linked to metabolic pathways involving iron-sulfur clusters, followed by emergence of methionine and AdoMet, then RNAs and RNA metabolism. A crucial step at this early stage was the RNA-dependent formation of polypeptides that ended up in the process of translation. A crucial family of associated functions was that of hydrolases, enzymes that often use Zn^{2+} at their catalytic centre. This ion would thus have gained its present role as an integrator of metabolism very early on. Highly specific chelators such as gliotoxin should help us investigate further in depth this scenario, which, if substantiated, will be critical for evolved metabolic engineering.

Conflict of interest

None declared.

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