Intracellular phase separation and its role in nickel sensing

(Article begins on next page)

1 **Intracellular phase separation and its role in nickel sensing**
2
3 Barbara Zambelli
4
5 Laboratory of Bioinorganic Chemistry, Department of Pharmacy and Biotechnology,
6 University of Bologna, Italy
7 barbara.zambelli@unibo.it
8
11
12
13 **Abstract**
14
15 Nickel homeostasis in many bacteria is controlled by the nickel-sensor NikR. A recent study
16 by Cao et al. found that *Escherichia coli* NikR undergoes phase separation and that this event
17 enhances its function as a nickel-dependent transcriptional repressor. The results suggest
18 that phase separation is functional for bacterial metal homeostasis.
19

20

Nickel features a dual nature of poisoning and essential element, being required for the biochemical reactions of many prokaryotes, unicellular eukaryotes, and plants. These organisms set up a tight balance between metal accumulation, distribution, and detoxification to control its intracellular quota. This task is usually carried out by specific transcription factors known as Ni(II)-sensors [1]. In *Escherichia coli*, nickel uptake and efflux are controlled by two distinct sensors, NikR and RcnR, respectively [2].

NikR is largely conserved in bacteria and archaea, being crucial for the virulence of pathogens as *Helicobacter pylori*, thus representing a target for antibacterial drug discovery. In *E. coli*, NikR functions as a repressor, binding to the promoter of the *nikABCDE* Ni(II)-import system when it is loaded with Ni(II). In the last two decades, several studies have investigated the mechanism through which NikR translates the intracellular Ni(II) concentration into a transcriptional response, based on structural, biophysical and functional data. They found that Ni(II) ions bind in a square-planar coordination geometry at the interface of a homo-tetrameric structure, made of the assembly of four C-terminal metal-binding domains (MBDs) (Figure 1A). At the opposite sides of the structure, two dimeric N-terminal DNA-binding domains (DBDs) are attached by flexible likers [3] (Figure 1A). In the apo-structure, different conformers (*open*, *cis* and *trans*) interconvert in solution, depending on the relative orientation of the DBDs to the MBDs (Figure 1B). Such binding heterogeneity is abrogated in the DNA-bound form, in which the DBDs are symmetrically locked to two major grooves in the *cis* orientation (Figure 1A,C) [2]. Protein-operator interaction requires Ni(II) binding, occurring with affinities in the nanomolar range (Figure 1C) [2]. Metal binding sites lie topologically far from the DBDs, thus Ni(II) binding should be allosterically propagated from the MBDs along the protein structure. This mechanism likely involves a modulation of the protein dynamics: Ni(II) binding does not shift the conformational landscape of the protein toward a single conformer, rather it likely slows down the mobility of the DBDs, increasing the time spent in a conformation competent for DNA binding, as suggested by experiments on *H. pylori* NikR [4].

A recent study published in *Cell Reports* [5] now adds a further layer to the mechanism of Ni(II)-dependent transcriptional regulation by NikR. Using fluorescence microscopy, Cao et al. found that *E. coli* NikR, reversibly undergoes concentration and temperature-dependent liquid-liquid phase separation (LLPS) in vitro. The C-terminal domains play a major role in this event, while the N-terminal domains only enhance the LLPS tendency of the full-protein [5]. Initially reported in eukaryotic cells, LLPS has been described as a cellular strategy for subcellular compartmentalization with dynamically forming phase-derived organelles. These biomolecular condensates are built by the co-localization of proteins, nucleic acids, and other regulating molecules [6]. More recently, some bacterial proteins were observed to undergo LLPS in vitro and in vivo, supporting a view of the bacterial cytoplasm far from an undifferentiated medium, rather organized in a structure that includes membrane-less organelles [7]. The ability of a protein to undergo phase separation in vitro is an important finding, but its physiological relevance should be proven in living cells, as in vitro systems cannot fully mimic the complexity of intracellular media [8]. To this aim, Cao et al. investigated the occurrence of NikR-driven LLPS in *E. coli*. An over-expressed fusion of GFP-NikR spatially localized near a pole region of the bacterial cells, hinting for the formation of intracellular condensates (Figure 1D). These assemblies were dissolved by 1,6-hexanediol (Hex), suggesting that they were liquid-like condensates and not solid-like aggregates [5].

Membrane-less organelles have the potential to be involved in many regulatory pathways, such as transcription, because they increase the local concentrations of all the

67 components involved, like enzymes, transcriptional regulators, and DNA operators. LLPS of
68 bacterial RNA polymerase was indeed reported in vitro and in cell, thus supporting the
69 physiological relevance of this mechanism in bacterial transcription [9]. Thus, how does the
70 observed LLPS impact on the role of NikR as a Ni(II)-dependent regulator? Cao et al. showed
71 that NikR does not need Ni(II) nor DNA to form LLPS and that Ni(II) moderately enhances the
72 formation of droplets in solution and in cell [5]. However, only the presence of Ni(II), and not
73 of other metal ions such as Zn(II) and Mn(II) able to bind the protein in vitro [10], allows the
74 co-localization of promoter DNA in the condensates, indicating that NikR response to Ni(II)
75 remains intact in the phase-derived structures [5]. Disruption of LLPS impaired the regulatory
76 function of NikR relieving the repression from the promoter and leading to an intracellular
77 Ni(II) overload and metal-toxicity [5]. These results hint to a functional role of LLPS in
78 enhancing the activity of NikR, thus required for bacterial resistance to high Ni(II)
79 concentrations, and are coherent with the previously observed ability of *E. coli* NikR to
80 increase its DNA binding affinity when Ni(II) is in excess [2].

81 The results shown in this study provide the first evidence that intracellular phase
82 separation might be functional to regulate Ni(II) ion homeostasis in bacteria. Further studies
83 are needed to understand how metal-driven changes in protein folding and dynamics
84 influence the formation of biomolecular condensates by NikR. As this protein is central for
85 metal homeostasis of several pathogens, this discovery, if confirmed for other NikR proteins,
86 might open new routes for drug discovery. It is noteworthy that the interaction of NikR with
87 Bi(III), a known drug against *H. pylori* that impairs bacterial Ni(II) homeostasis, prevents LLPS
88 by NikR both in solution and in cell [5].

89 The discovery that a bacterial metal-sensor is regulated by LLPS opens new
90 perspectives and will likely stimulate the research, with the evaluation of this event in other
91 metal-dependent systems. Some examples of metal-induced LLPS were reported in the past,
92 but they do not involve physiological processes carried out by metallo-proteins. Considering
93 that one third of all existent proteins require transition metal ions for their function we can
94 envisage that membrane-less organelles that contain metallo-proteins might regulate other
95 physiological processes, possibly influenced by intracellular metal availability.

96
97 **Figure legend.**

98
99 **Figure 1. NikR forms biomolecular condensates in *E. coli* cells.** A) *E. coli* NikR binds to the
100 *nik* operator DNA in a homo-terameric structure (PDB: 2HZV) binding four Ni(II) ions at the
101 tetrameric interface (details in the insert). B) Different conformers of NikR interconverts in
102 solution depending on the position of the DNA binding domains connected to the central
103 metal binding domains by flexible linkers. C) In the presence of Ni(II), NikR binds the *nik*
104 promoter DNA and represses the transcription of *nikABCDE* Ni(II) uptake system. D)
105 Condensates of *E. coli* NikR accumulate in a pole of the bacterial cells and regulate the
106 activity of NikR as a Ni(II)-sensor. This figure was created using BioRender
107 (https://biorender.com/). Representation of the protein structure was created using 3D
108 Protein Imager (https://3dproteinimaging.com/protein-imager/).
109
110
111
112
113

References

1. Zambelli, B. et al. (2012) Metal ion-mediated DNA-protein interactions. Met Ions Life Sci 10, 135-70.

2. Musiani, F. et al. (2015) Nickel-responsive transcriptional regulators. Metallomics 7 (9), 1305-18.

3. Schreiter, E.R. et al. (2006) NikR-operator complex structure and the mechanism of repressor activation by metal ions. Proc Natl Acad Sci U S A 103 (37), 13676-81.

4. Baksh, K.A. et al. (2021) Allosteric regulation of the nickel-responsive NikR transcription factor from Helicobacter pylori. J Biol Chem 296, 100069.

5. Cao, K. et al. (2023) Cellular uptake of nickel by NikR is regulated by phase separation. Cell Rep 42 (6), 112518.

6. Banani, S.F. et al. (2017) Biomolecular condensates: organizers of cellular biochemistry. Nat Rev Mol Cell Biol 18 (5), 285-298.

7. Greening, C. and Lithgow, T. (2020) Formation and function of bacterial organelles. Nat Rev Microbiol 18 (12), 677-689.

8. Alberti, S. et al. (2019) Considerations and Challenges in Studying Liquid-Liquid Phase Separation and Biomolecular Condensates. Cell 176 (3), 419-434.

9. Ladouceur, A.M. et al. (2020) Clusters of bacterial RNA polymerase are biomolecular condensates that assemble through liquid-liquid phase separation. Proc Natl Acad Sci U S A 117 (31), 18540-18549.

10. Wang, S.C. et al. (2004) Selectivity of metal binding and metal-induced stability of Escherichia coli NikR. Biochemistry 43 (31), 10018-28.

(A) MBDs DBDs DBDs Cys95 His76' His87 His89

(B) Cis Open Trans

(C) Ni(II) *nikABCDE* RNA pol.

(D)