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Commentary

Mg^{2+} ions mediate the interaction of intrinsically disordered nascent chains with the ribosome: implications for protein folding and aggregation in the early stages of protein life

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During the last few decades, the ribosome has been regarded primarily as a major cell player devoted to the catalysis of protein biosynthesis during translation [1-5]. It is therefore not surprising that several processes related to translation exploit the ribosome as a central hub. For instance, it is well-known that many events related to translational regulation are mediated by interactions between the ribosome and initiation, elongation or termination factors [6-9]. In addition, the ribosome is involved in mRNA-code recognition and proofreading [10-12] as well as in the control of translation rates via interactions with mRNA codons bearing high- and low-frequency [13-15] and associated with variable tRNA abundance within the translation machinery [16-18]. Interestingly, the ribosome also assists *de novo* protein structure formation by minimizing cotranslational aggregation, thus increasing the yield of native-protein production [19,20]. The latter event, however, has not been shown to require -- or even involve -- direct interactions between the ribosome and the nascent protein chain. A notable exception is that of nascent chains bearing either N-terminal signal sequences or translational-arrest tags. These proteins are known to establish short- or long-term contacts with various regions of the ribosome during translation [21-25]. In summary, until recently very little knowledge has been available about direct contacts between the ribosome and nascent polypeptides and proteins that do not carry signal or arrest sequences. Studies based on fluorescence depolarization in the frequency domain [26] and NMR spectroscopy [27-30] provided interesting data that are consistent with, but do not unequivocally establish, the presence of these interactions.

A recent investigation by Guzman-Luna *et al.* [31] added new knowledge to the field by showing that two bacterial ribosomal proteins, L23 and L29, interact with ribosome-bound nascent protein chains (RNCs) encoding an intrinsically disordered protein (IDP) and several of its mutants bearing variable net charge. This study is based on site-specific fluorescence labeling of RNCs at their N terminus, in combination with chemical crosslinking and Western blotting. Intriguingly, short RNCs were found to interact only with the L23 ribosomal protein while longer RNCs also weakly interact with an additional ribosomal protein, L29. The interacting proteins are located within a specific region of the large ribosomal subunit, which comprises the vestibule of the exit tunnel as well as the immediately adjacent outer region of the ribosome.

Importantly, a large fraction of the detected interactions is mediated by Mg^{2+} ions, and the extent of the contacts can be tuned up and down depending on the Mg^{2+} concentration in the medium [31]. Interestingly, Mg^{2+} ions have been long known to be ribosome-associated [32,33] and to play a key role in preserving the structural integrity of the ribosome [32,33]. However, no Mg^{2+}-mediated processes involving nascent proteins were ever detected before the work by Guzman-Luna *et al.* In summary, the study by Guzman-Luna *et al.* [31] highlights a new role for the ribosome, which is found to interact with intrinsically disordered nascent chains in a Mg^{2+}-mediated fashion.

On the other hand, some (ca 50%) of the interactions of nascent proteins with the ribosome were found to be Mg^{2+}-independent. Based on a quantitative electrostatic mapping of the ribosomal surface, which contains a large nonpolar patch on the solvent-exposed region of L23 within the exit region of the ribosomal tunnel [31], it is possible that the Mg^{2+}-independent interactions are dominated by the hydrophobic effect [34]. Some classical electrostatic interactions not involving Mg^{2+} may also be present. However, these types of interactions are surprisingly not dominant. Ongoing and future studies will shed additional light on the remaining unanswered questions regarding the detailed nature of these IDP-ribosomal-protein interactions.

The study by Guzman-Luna and coworkers [31] nicely complements the current knowledge on the effect of Mg^{2+} ions on non-ribosome-related functions. For instance, Mg^{2+} is known to play a seminal role in bacterial thermodynamic balance [35-37] and in proteostasis, including modulating the activity of the Hsc62 [38] and Hsp70 [39] molecular chaperones and altering the specificity of the Lon and ClpAP proteases [40].

A few major lingering questions are (a) whether the above interactions are also present in the case of proteins with a foldable sequence, as opposed to IDPs, (b) what the role of the Mg^{2+}-dependent and Mg^{2+}-independent ribosome-RNC interactions is, and (c) whether similar interactions are also present across eukaryotic organisms. Ongoing research in the Cavagnero group (Guzman-Luna *et al.*, manuscript in preparation) suggests that extensive ribosome-RNC interactions are also present to in the case of foldable protein sequences. However, their ion dependence is different and much more complex.

On a different note, while analogous studies on eukaryotic organisms are not available yet, it is notable that intracellular Mg^{2+} concentration is known to be important for human brain cytopathies [41], and that the concentration of Mg^{2+} ions has an effect on the extent of post-translational protein aggregation, as recently found in the case of TDP-43 [42]. Further, several known proteinopathies are related to the misfolding and aggregation of intrinsically disordered polypeptides and proteins [43,44]. It is also worth noting that the prokaryotic and eukaryotic ribosomes share a similar structure and overall highly negative electrostatic surface potential [45]. Incidentally, the ribosome (or fragments thereof) has been previously proposed to serve as a chaperone [46,47]. Therefore, despite the undeniable differences between ribosomes in all classes of organisms [6,48], it is tempting to hypothesize that Mg^{2+}-mediated interactions between intrinsically disordered nascent proteins and the ribosome may play a role in regulating protein quality in bacteria and perhaps even in higher organisms. The known Mg^{2+} dependence of translation efficiency is consistent with this hypothesis [37].

In conclusion, Mg^{2+} mediates the interaction of intrinsically disordered nascent proteins and the bacterial ribosome [31]. Future studies will reveal whether Mg^{2+}-mediated RNC-ribosome contacts influence the quality of *de-novo* synthesized proteins in bacteria. In the case of higher organisms, if confirmed to be present, ribosome-RNC interactions may influence the course of diseases that critically depend on the *de novo* generation of proteins devoid of aggregation. While more studies clearly need to be carried out to unequivocally identify additional trends, the present findings promise exciting opportunities for future discovery.

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