In-cell integrative structural biology of transcription-translation coupling

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Abstract

Bridging scales from atomic resolution to entire cells is a technical and computational challenge. Over the past decade, crosslinking mass spectrometry (MS) has developed into a robust and flexible tool that provides medium-resolution structural information (1). Crosslinking MS data provide a measure of the proximity of amino acid residues and thus offer information on the folds of proteins and the topology of their complexes. Pairing Crosslinking MS with the remarkable advances of electron tomography and integrative modeling opens up the possibility of studying cellular processes in situ. We developed such an integrative in-cell structural approach using the genome-reduced human pathogen Mycoplasma pneumoniae to determine an in-cell architecture of a transcribing and translating expressome at subnanometer resolution (2). The expressome comprises RNA polymerase (RNAP), the ribosome, and the transcription elongation factors NusG and NusA. We pinpointed NusA at the interface between a NusG-bound elongating RNAP and the ribosome and propose that it can mediate transcription-translation coupling. Translation inhibition dissociated the expressome, whereas transcription inhibition stalled and rearranged it. Thus, the active expressome architecture requires both translation and transcription elongation within the cell. These findings highlight the enormous potential of integrative in-cell structural biology approaches in elucidating dynamic and complex cellular processes within their native context.

References:

1) Cross-linking mass spectrometry: methods and applications in structural, molecular and systems biology. O'Reilly FJ, Rappsilber J. Nat Struct Mol Biol. 2018 Nov;25(11):1000-1008.
2) In-cell architecture of an actively transcribing-translating expressome. [O'Reilly FJ, Xue L, Graziadei A], Sinn L, Lenz S, Tegunov D, Blötz C, Singh N, Hagen WJH, Cramer P, Stülke J, Mahamid* J, Rappsilber* J. Science. 2020 Jul 31;369(6503):554-557. [] joined first authors, * communicating authors