Seminar

Dr. Sarah Loerch

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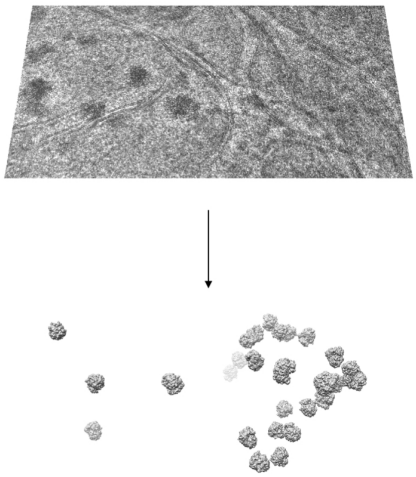
"Structural basis of P-body regulation in sensory neurons"



Processing bodies (p-bodies) are prototypical phase-separated RNA containing granules which are enriched for factors implicated in RNA metabolism. In mammals, their physiologic functions are largely unknown. In immortalized cell lines, p-bodies are thought to store mRNAs as they increase in abundance when translation is compromised. We sought to determine if the mechanisms that govern p-body abundance are similar in primary mouse sensory neurons. Unlike their somatic counterparts, pharmacologic blockade of translation initiation has no effect on sensory neuron p-bodies (SNPBs). However, inhibition of translation elongation factor eEF2 via eEF2K leads to a profound reduction SNPBs in sensory neurons while having no effect on p-body abundance in cell lines. To better understand how eEF2K inhibition impacts translation, we isolated ribosomes from treated neurons. Structural analyses revealed a large population of 80S ribosomes containing SERBP1 in place of mRNA and phosphorylated eEF2 bound to the acceptor site. We conclude that eEF2K plays a key role in SNPB dynamics and that agonists of eEF2K stabilize a vacant conformation of the 80S ribosome. As an initial step towards investigating these distinct ribosomes in their native environment – the neuron – we developed a method using high-resolution template-matching cryo-EM to quickly identify large numbers of individual ribosomes and determine their translation activity. We validated this approach using crudely purified translating ribosomes that were stalled with an antibiotic that targets elongating (translating) ribosomes. Comparison of the results with the occupancy of individual ribosomes in single-particle classes obtained from the same data showed correctly identifies the activity of a large number of individual ribosomes. We can now spatially map and identify the translation activity of 80S ribosomes in neuronal processes.

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Hosted by Stephen Floor