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Sleeping Beauty Redux – Resting Ribosomes in Neurons

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Plasticity is central to cellular function. Neurons leverage extracellular cues within their microenvironments to regulate local protein synthesis and facilitate plasticity. For example, dynamic and localized protein production in response to stimuli like neurotransmitter exposure, termed activity-dependent translation, mediates the remodeling of the postsynaptic neuron. Cells deposit specific RNAs (1, 2), proteins (3), and translation machinery (4) in discrete locations where they await activation. Even though it is known that localized translation facilitates plasticity, a mechanistic understanding of location-dependent ribosome regulation is lacking due to a lack of high-resolution methods. Questions such as: what inactivates ribosomes? And how are they reactivated remain largely unanswered.

We recently found that a kinase, eukaryotic elongation factor 2 kinase (eEF2K), attenuates translation in sensory neurons by creating a reservoir of idle but fully assembled 80S ribosomes (5). Using single-particle cryogenic electron microscopy (cryo-EM), we found that these ribosomes are bound to the phosphorylated eEF2K target, eukaryotic elongation factor 2 (eEF2) and a protein, named SERBP1, in the mRNA channel. Both eEF2 and eEF2K have a known yet poorly understood role in activity-dependent translation. We hypothesize that these bound proteins regulate ribosome localization and that these resting ribosomes are reactivated by upstream signals.

As an initial step towards investigating the function of eEF2-bound resting ribosomes in cells, we leverage a new *in situ* cryo-EM method termed 2D template matching (2DTM) (6, 7). 2DTM uses high-resolution molecular templates to identify their location and orientation in cells from untilted 2D images. To validate this approach, we used 2DTM with templates of rotated and non-rotated 80S ribosome conformations on crudely purified translating ribosomes that were stalled with an antibiotic that targets elongating (translating) ribosomes. We compared the 2DTM results with the occupancy of individual ribosomes in single-particle classes obtained from the same data. We conclude that this approach correctly identifies the activity of most individual ribosomes. Using HRTM, we now can spatially map and identify the translation activity of 80S ribosomes in neuronal processes of rat neurons grown on cryo-EM grids.

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