Ribosome standby: How bacteria translate proteins from structurally blocked mRNAs

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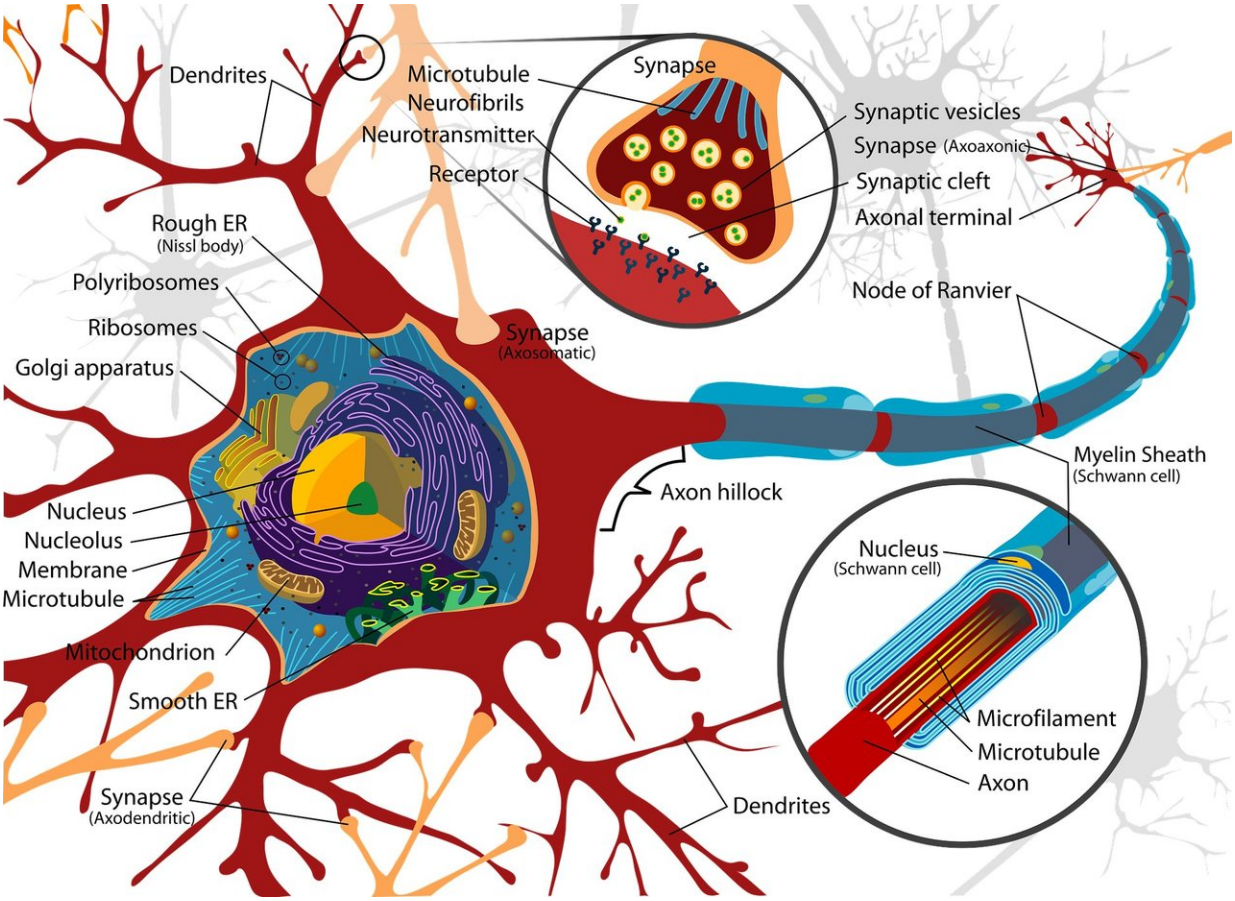

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Bacterial ribosomes need a single-stranded ribosome binding site (RBS) to initiate protein synthesis, whereas stable RNA structure blocks

initiation. Paradoxically, structured mRNAs can nevertheless be efficiently translated. Researchers at Uppsala University have now elucidated the anatomy of a "standby" site and its requirements, to overcome RNA structure problems for translation.

Bacterial protein synthesis has been studied for decades. Ribosomes needs access to a single-stranded RBS to initiate translation. However, some mRNAs with stably structured RBS regions are efficiently translated. About 25 years ago, Dutch researchers proposed a new mechanism to account for this, "ribosome standby": a ribosome binds to an accessible, unstructured region elsewhere, waits for a while, and then moves to the RBS when its structure temporarily opens.

In this new study, published in *Proceedings of the National Academy of Sciences*, researchers at Uppsala University have unveiled the anatomy of a standby site, and reported on the key role of ribosomal protein S1 in this process. S1 binds to a standby site consisting of two elements, a single-stranded region and—unexpectedly—a short RNA hairpin. Standby binding permits the ribosome to move through downstream RNA structure and to access the blocked RBS.

"We felt that it was time to figure out what exactly a standby site looks like, and what is needed to make it work. Standby is an old idea that up to now lacked strong direct evidence," says Cédric Romilly, the study's first author.

Following studies conducted by the Wagner group for years, they investigated a short mRNA that encodes a toxin, TisB. Translation of this protein is entirely dependent on a standby site located >100 nucleotides upstream of the stable and inaccessible RBS structure. Using sophisticated biochemical methods, such as fluorescence anisotropy and UV-crosslinking/ RNA footprinting, the researchers were able to catch the ribosome on the standby site. The experiments show that it is protein

S1 that guides the ribosome to the standby site, thus likely promoting downstream RNA structure opening to access the TisB RBS.

"This has really been a tour de force, but it is great to finally understand the anatomy of a real standby site," says Professor E. Gerhart H. Wagner, lead author of the study.

More information: Cedric Romilly el al., "The ribosomal protein S1-dependent standby site in tisB mRNA consists of a single-stranded region and a 5′ structure element," *PNAS* (2019). www.pnas.org/cgi/doi/10.1073/pnas.1904309116

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