terial surface polysaccharides such as lipopolysaccharide and peptidoglycan (*12*, *13*). Thus, Jones *et al*. have added noroviruses (*Caliciviridae*) to this list of enteric viruses that benefit from bacteria. Validation of this promising new cell culture model for human norovirus infection will pave the way for studies that have been impossible for decades.

A common theme is emerging: Enteric viruses bind to bacterial surface glycans, which directly or indirectly promotes viral replication. There are several questions that emerge from the findings of Jones *et al*. relevant to this theme. It is not clear how bacterial histo-blood group antigens enhance human norovirus attachment to B cells. Studies on poliovirus suggest that bacterial surface glycans enhance the binding of virions to the viral receptor protein (*13*). Perhaps histo-blood group antigen-bound noroviruses have enhanced attachment to host cell glycans or another cellular cofactor. There is also the question of whether additional human norovirus genotypes infect B cells, and whether bacterial histo-blood group antigens are required. There are more than 25 highly variable human norovirus genotypes. The binding site for histo-blood group antigens on viral particles is hypervariable and it is reasonable to expect differences in antigen-binding affinity.

Do bacteria promote norovirus infections in humans? If so, how prevalent are bacteria that express histo-blood group antigens in the human intestine, and do some of those strains promote human norovirus infection more than others? Microbial communities vary from person to person. It is possible that some individuals harbor more bacterial strains that promote human norovirus infection than others.

We are only beginning to understand the roles of commensal bacteria in the human gut for viral pathogenesis and/or transmission. Future work in this area may yield novel strategies for treating enteric virus infection and limiting transmission.

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CELL BIOLOGY

Local synthesis and disposal

New experimental strategies reveal spatial and temporal features of protein synthesis and degradation in cells

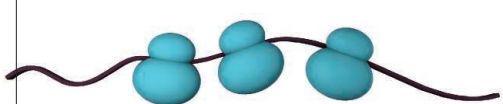
By **Sichen Shao** *and* **Ramanujan S. Hegde**

Most cells in our body have functionally specialized regions containing distinct sets of proteins. The evolution of this complexity from prokaryotic ancestors relied on the capacity to generate, sustain, and regulate subcellular compartments. At least part of this specialization exploited the ability to synthesize and degrade subsets of proteins in restricted areas of the cell. For example, neurons change the responsiveness of individual synapses during learning by controlling local protein production and degradation (*1*). Discovering and analyzing such local pathways amid parallel general pathways has posed a substantial challenge. On pages 751, 716, and 748 of this issue, Foresti *et al*. (*2*), Jan *et al*. (*3*), and Williams *et al*. (*4*), respectively, make progress toward this aim by applying powerful whole-proteome analyses in a yeast model, discovering a new pathway for localized protein degradation (*2*) and providing unprecedented views of all protein synthesis occurring at particular organelles (*3*, *4*). The findings may guide the study of region-specific reactions in morphologically complex metazoan organisms.

Foresti *et al*. and Jan *et al*. focus on the endoplasmic reticulum (ER), an expansive and functionally diverse eukaryotic organelle. This is the site of maturation for nearly all secreted and integral membrane proteins, collectively representing ~20% of all genes in a typical eukaryotic genome. Proteins that fail to assemble properly are destroyed by ER-associated protein degradation (ERAD). The diversity of clients transiting through the ER means that biosynthesis and degradation must be remarkably pliant. Hence, neither process occurs through a unifying pathway; rather, multiple routes, differing in their clientele, collectively handle this diversity (*5*, *6*). These pathways have been characterized for a handful of proteins, but the overall flux through each pathway has been difficult to infer without broader analyses.

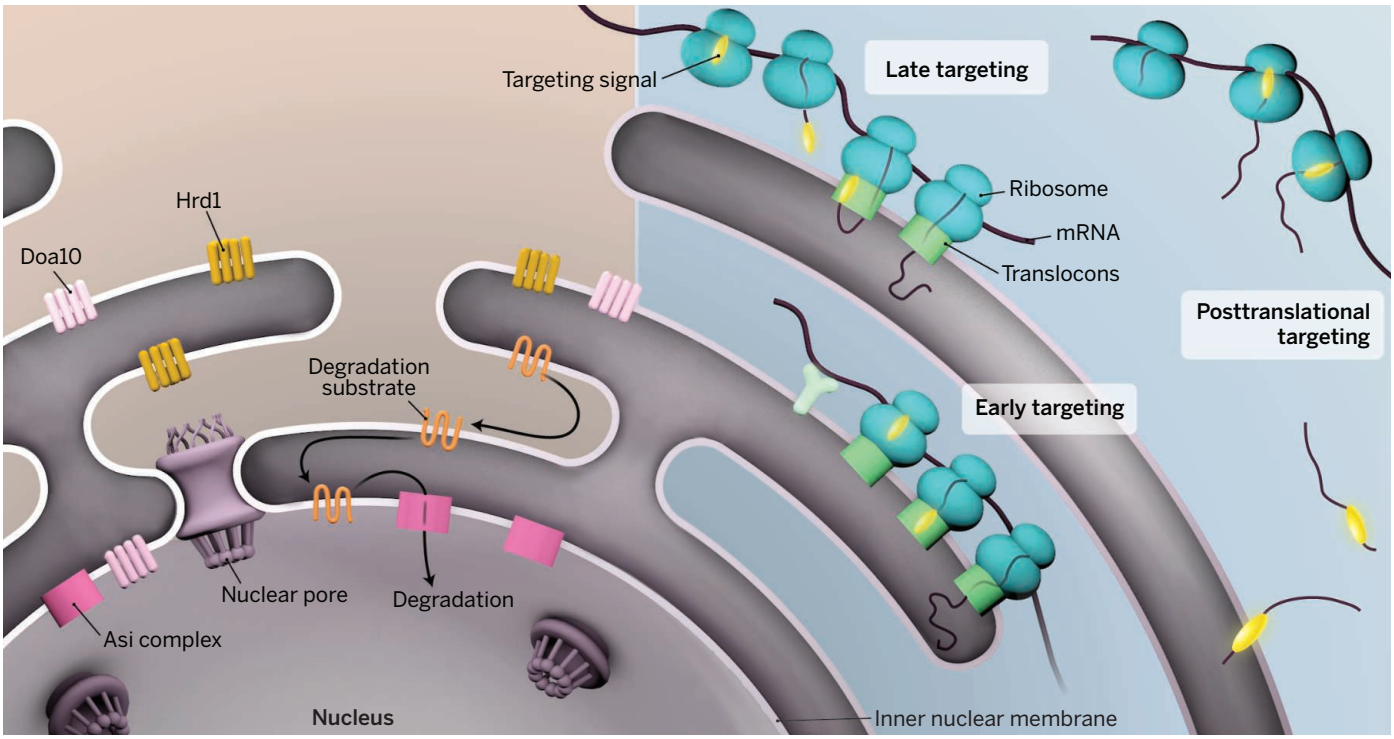
Foresti *et al*. exploit proteome-wide tracking of protein abundances to detect a new degradation pathway. In yeast, ERAD was thought to comprise two pathways defined by ubiquitin ligases (Hrd1 and Doa10) that polyubiquitinate proteins for degradation (*6*). These ligases use ubiquitin supplied by the factor Ubc7. Using a quantitative proteomics method (*7*), the authors identified substrates whose degradation requires Ubc7 but neither ligase. Following up one substrate, Erg11, led to the discovery of a new ERAD pathway that uses the Asi ubiquitin ligase complex (see the figure). The Asi complex localizes to the inner nuclear membrane, a restricted subdomain of the ER that was not previously appreciated to have membrane protein quality control. The amount of Erg11 protein, an enzyme for sterol synthesis, could perhaps be regulated by controlling its trafficking to the inner membrane.

Although sequence homologs of the Asi complex are not apparent outside fungi, the findings of Foresti *et al*. will stimulate a search for the analogous pathway in other organisms. It is noteworthy that, unlike the traditional strategy of using a model protein to drive pathway discovery, a global search for clients that do not fit into existing frameworks pointed the way to both a new pathway and an ideal model substrate for its analysis.



The power of quantitative proteomics may well shift how new biological pathways are uncovered in future studies.

This is why the study by Jan *et al*., describing strategies to obtain global views of localized protein synthesis, is an important conceptual and technical advance. Ribosome profiling was previously described as a means to quantitatively identify ribosomal footprints on all cellular mRNAs—snapshots of the global translational program—under different conditions (*8*). Jan *et al*. sought to refine ribosome profiling by analyzing a localized subset of ribosomes. The authors pursued an orthogonal tagging strategy (*9*) in which a peptide acceptor sequence becomes biotinylated when it encounters the biotin ligase BirA. Such tagging has been used to infer protein-protein interactions (*10*) and protein exposure to specific cellular compartments (*11*). Thus, incorporating the biotin acceptor site into a ribosomal protein and localizing BirA to a specific cellular area allows tagging of only those ribosomes in proximity to BirA. Tagged ribosomes can then be purified and analyzed to determine



Spatial specialization. (**Left**) Different degradation machineries in the ER membrane have distinct localizations. The Asi complex, which tags proteins destined for destruction with ubiquitin, is restricted to the inner nuclear membrane. Its substrates must first enter the nucleus. (**Right**) The timing of ribosome engagement with ER translocons is highly variable.

their position along individual mRNAs.

Jan *et al.* develop this strategy for the ER membrane. Many secretory and integral membrane proteins are coordinately translated and inserted into the ER at translocons, conduits across or into the membrane. Ribosomes translating these proteins were preferentially biotinylated by ER-tethered BirA, whereas BirA tethered at mitochondria labeled ribosomes translating mitochondrial proteins. Ribosomes translating tail-anchored proteins, which target to the ER after translation (*12*), were not tagged by ER-localized BirA. Thus, proximity-based ribosome profiling is a minimally perturbing method for identifying all proteins produced at a specific location, permitting Jan *et al.* to define putative secretory proteins not predicted by bioinformatics alone.

The same strategy was applied by Williams *et al.* to define proteins imported into mitochondria, clarifying numerous ambiguous bioinformatic annotations. Curiously, the fumarate reductase Osm1 seemed to be synthesized at both mitochondrial and ER membranes. By analyzing the ribosomal start site footprints, they identified alternative translation initiation as the mechanism of this dual localization. Williams *et al.* noticed that enrichment of most mitochondrial clients was only seen when translation was stalled for several minutes prior to analysis, indicating that they are ordinarily synthesized on cytosolic ribosomes and translocated after translation. Thus, manipulating translation elongation allowed Williams *et al.* to discriminate between posttranslational and cotranslational routes, revealing that mitochondrial inner membrane proteins preferentially use the latter.

Proximity-specific ribosome profiling also allows analysis of how individual substrates engage their translocon. In yeast, ribosomes can use one of two homologous ER translocons built around either Sec61 or Ssh1; the former can also associate with the Sec63 complex to mediate posttranslational translocation (*5*). By fusing BirA to either Sec63 or Ssh1, Jan *et al.* aimed to identify their respective clientele. Although most clients promiscuously engage both proteins, the respective profiles of ribosome footprints obtained with Ssh1- and Sec63-tethered BirA showed some curious differences. In general, the time of ribosome arrival at a translocon, marked by where mRNA footprints are first seen, occurs just as the signal for targeting to the translocon emerges from the ribosome. However, some Sec63-marked clients arrive at the translocon earlier, perhaps indicating that the respective mRNAs were pre-tethered to the ER membrane. By contrast, some Ssh1-marked clients arrive later, possibly reflecting a need for extra nascent chain length for productive insertion into the translocon. Although the basis of these observations is speculative, they highlight the timing of ribosome targeting as a variable that merits future investigation.

Tuning the promiscuity of BirA (*10*), adapting it to use biotin analogs (*13*), and developing variants that can be activated and inactivated will provide greater spatial and temporal control of the proximity-specific tagging method, a likely prerequisite for its adaptation to more complex organisms. Combining this with genome editing and the increasing ability to produce diverse cell types in culture should provide opportunities to explore spatially regulated protein dynamics in numerous physiological contexts. ■

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