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SPOTLIGHT

A TRCky TA protein delivery service snubs the UPS

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In mammals, tail-anchored (TA) proteins that are posttranslationally captured by the chaperone SGTA are triaged by the BAG6 complex into one of two fates: handoff to an ER targeting factor for membrane insertion or polyubiquitination for destruction by the proteasome. In this issue, Culver and Mariappan (2021. *J. Cell Biol.* https://doi.org/10.1083/jcb.202004086) show that a fraction of newly synthesized TA proteins is polyubiquitinated in HEK293 cells independently of the BAG6 complex yet evades proteasomal degradation by undergoing deubiquitination en route to becoming stably inserted into the ER membrane.

Nascent membrane proteins must avoid aggregation of their hydrophobic transmembrane domains (TMDs) in the aqueous cytosol before insertion into the lipid bilayer. Signal recognition particle (SRP) solves this problem for the majority of membrane proteins destined for the secretory pathway by shielding their N-terminal hydrophobic regions shortly after they emerge from the ribosome exit tunnel. SRP also pauses translation until the ribosome is directed to the ER protein translocation channel Sec61, such that subsequently synthesized TMDs reach the lipid bilayer with minimal exposure to the cytosol. By contrast, the single C-terminal TMD of tail-anchored (TA) proteins emerges from the ribosome exit tunnel after translation has terminated, necessitating a distinct pathway for posttranslational TA protein targeting and insertion into the ER membrane. In mammals, this is accomplished by the TMD recognition complex (TRC) pathway comprising chaperones, an ER targeting factor, and a dual ER transmembrane receptor and insertase (Fig. 1; 1).

The initial fate of TA proteins as they emerge from the ribosome exit tunnel has been an area of intense investigation over the past decade. Early attempts at identifying the pre-targeting factors in mammalian cells led to a model in which a complex containing BAG6, TRC35, and Ubl4A is recruited to the ribosome to chaperone emerging TA proteins and direct them to the ER targeting factor TRC40 (2, 3). Subsequent work demonstrated that the chaperone SGTA (small glutamine-rich tetratricopeptide repeat-containing protein α) captures nascent TA proteins prior to interaction with the BAG6 complex, which scaffolds the SGTA–TA complex, TRC40, and the E3 ubiquitin ligase RNF126 (4, 5, 6). At this stage, the destiny of TA proteins can be bifurcated: either they are successfully transferred to TRC40, thus committing them for targeting to the ER membrane and insertion by tryptophan-rich basic protein and calcium-modulating cyclophilin ligand (WRB/CAML; the TRC40 receptor and TMD insertase), or they are handed over to BAG6 for polyubiquitination by RNF126, leading to their proteasomal degradation (5). SGTA is additionally thought to regulate degradation of TA proteins by antagonizing BAG6-mediated ubiquitination (7).

Notably, the work that identified the mechanism by which BAG6 helps TRC40 avoid substrate overload relied mostly on in vitro systems but didn't explore fully the cell dynamics of nascent TA proteins with the ubiquitin proteasome system (UPS; 3, 4, 5, 6, 7). In this issue, Culver and Mariappan use a series of cell-based assays to demonstrate that following synthesis a fraction of TA proteins gets modified with K48-linked polyubiquitin chains in the cytosol yet fails to be destroyed as a typical UPS substrate (8). Surprisingly, this fraction was neither affected by the presence of excess SGTA nor the absence of BAG6. Even more heretically for the elegant triage view by which the TRC pathway intersects with the UPS, the polyubiquitinated fraction was competent for interaction with TRC40 and became successfully inserted into the ER membrane. Finally, the authors showed that some TA protein deubiquitination occurs concomitantly with insertion and identified a pair of ER-localized deubiquitinases (DUBs), USP20 and USP33, that are necessary and sufficient to fully complete pruning after insertion has taken place.

The identity of the ubiquitination machinery defined by this work remains unknown, which raises questions about its mechanism and logic. The authors detect that ~8% of nascent TA proteins become polyubiquitinated dependent on the presence of a TMD, but acknowledge that polyubiquitination should occur on the cytosolic domains of TA proteins whose TMDs lack lysine residues. However, it's unclear if the ubiquitination machinery has a bona fide TMD-recognition factor (akin to BAG6) or instead engages clients with more eclectic hydrophobic regions. Indeed, around 12–15% of all nascent polypeptides are co-translationally ubiquitinated in HEK293T cells (9). Thus, a parsimonious working model would be that the TMD of a polyubiquitinated TA protein still enables rapid transit along the TRC

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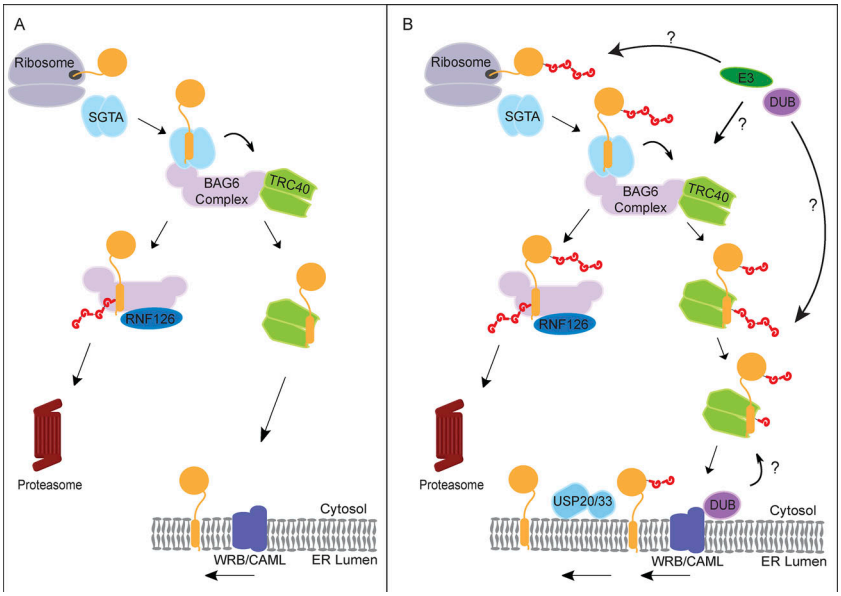

Figure 1. **How TA protein biogenesis is embedded within the UPS. (A)** In the canonical model, TA proteins that fail to be transferred to TRC40 are captured by BAG6 and polyubiquitinated by RNF126 prior to proteasomal degradation. **(B)** The work of Culver and Mariappan supports an alternative model in which polyubiquitination of TA proteins by undefined E3 ligase(s) occurs earlier in their biogenesis but does not preclude capture by TRC40 or insertion into the ER by WRB/CAML. Undefined DUBs counteract the E3 activity at many steps prior to insertion, after which USP20/33 finally removes all residual ubiquitin.

pathway all the way to the ER DUBs at the end of the line. Such a sequence of events makes for a messier textbook picture, but might allow for more efficient biogenesis of TA proteins than when innocent bystanders are destroyed.

Is reversible polyubiquitination genuinely futile or part of an as yet undefined quality control system? The authors speculate that ubiquitination of nascent TA proteins could serve to cage their soluble domains against promiscuous interactions or activity en route to their final destinations. Future identification of the E3 ligase(s) responsible will provide a much-needed handle for testing this idea and other whimsical possibilities. Going beyond HEK293T cells might of course also reveal a cellular context in which this TA protein ubiquitination process leads to a degradation fate.

Lastly, it will be important to identify the DUBs that apparently generate trimmed TA protein substrates for USP22/30. Is their DUB activity coupled to earlier steps of the TRC pathway or simply coincident with TA protein targeting and insertion? More broadly, the work of Culver and Mariappan illustrates the power of "old-school" cell-based approaches to reveal new insights into membrane protein biogenesis. Running their playbook against posttranslational membrane protein targeting pathways to mitochondria and peroxisomes will either extend their dynamic ubiquitination view into a paradigm or at the very least require that we turn another textbook picture on a dime.

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