Published in final edited form as:

Trends Biochem Sci. 2014 April ; 39(4): 154–156. doi:10.1016/j.tibs.2014.02.001.

Defective secretory-protein mRNAs take the RAPP

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Abstract

Secretory proteins destined for the lumen of the endoplasmic reticulum are key regulators of cellular functions and are thus subject to several levels of quality control. A recent study finds that the earliest step in secretory protein biogenesis, i.e. binding of the signal recognition particle to the signal sequence of the nascent peptide, is subject to a quality control process termed RAPP for “regulation of aberrant protein production”. This process involves AGO2 and mRNA degradation.

Keywords

Signal recognition particle (SRP); endoplasmic reticulum; Argonaute 2 (AGO2); mRNA degradation; secretory protein; signal sequence; regulation of aberrant protein production (RAPP); quality control

Quality control pathways are essential for maintaining cellular homeostasis and are thus set in place at nearly every point from the conversion of hereditary material (DNA) into messenger RNA (mRNA) through to the conversion of mRNA into cellular protein. Although many proteins remain in the cytosol to execute their functions, a large class of proteins is destined for the secretory pathway. This class of proteins presents a topological conundrum for the cell: although synthesized in the intracellular environment (cytosol), they must be translocated into the endoplasmic reticulum (ER) lumen, secreted outside of the cell, or integrated into a membrane.

Mammalian cells have solved this problem through specialized localization sequences fused to the N-terminus of most soluble secretory proteins. These signal sequences consist of at least one positively charged amino acid followed by a stretch of hydrophobic residues. As the signal sequence is synthesized and the nascent polypeptide chain exits the ribosome, it is bound by a ribonucleoprotein complex called the signal recognition particle (SRP), which arrests translation and delivers the ribosome/mRNA/nascent peptide to the SRP receptor embedded within the ER [1] (Figure 1, top). With the aid of GTP hydrolysis, the ribosome/mRNA/nascent peptide is transferred to the “translocon”, a proteinaceous channel through

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which the nascent peptide passes as the ribosome resumes synthesis, ensuring that the final product is properly localized to the ER lumen. When its job as a localization signal is completed, the signal sequence is cleaved from most secretory proteins by an ER-associated signal peptidase. Considering the many regulated steps along the way, it is clear that much can go wrong during this process, making quality control processes essential. Among the most basic steps that should be monitored during the biogenesis of secretory proteins is the interaction between their nascent signal sequence and SRP. Since most secretory proteins fundamentally differ from cytoplasmic proteins by more than the presence of a signal sequence, loss of this interaction could in theory relegate synthesis of a secretory protein to the reducing environment of the cytosol, where disulfide bond formation and N-linked glycosylation (both common to most secretory proteins) cannot occur, resulting in misfolded proteins that are ultimately deleterious to the cell. With this logic in mind, the authors of an exciting recent study describe a pathway that ensures the fidelity of the cotranslational ER import of secretory proteins by monitoring the SRP-signal sequence interaction [2].

As a point of departure, Karamyshev et al. systematically deleted hydrophobic residues from the model secretory protein preprolactin (PPL). The result was a decrease in levels of protein produced, which was surprisingly due not to proteasomal destruction of the protein, but to a reduction in the steady-state levels of the mRNA from which the protein derived. This is a general phenomenon because mutations to the natural signal sequences of $\alpha 1$ -antitrypsin (AT) and carbonic anhydrase IV (CA4) yielded similar results despite differences in their signal sequences and the protein product encoded by the template mRNA. To pinpoint at what step during the biogenesis of a secretory protein this mRNA reduction occurs, the authors utilized small interfering RNA (siRNA)-mediated depletion of SRP, the SRP receptor subunits, or the translocon. Of these, only depletion of SRP yielded a reduction in the levels of both wild-type and mutated PPL mRNA. This was further accompanied by a reduction in the levels of mRNAs encoding endogenous secretory proteins, strongly implicating the interaction of SRP with a signal sequence as the step that determines the fate of the encoding mRNA. Using a Tet-Off system to attenuate transcription, the authors found that the observed reduction in steady-state mRNA levels is due to mRNA degradation.

Several quality control pathways that operate at the level of the ER have already been described. The unfolded protein response (UPR), for example, monitors the folding status of ER proteins. When misfolded proteins accumulate to unacceptable levels, the UPR initiates a program of general translational attenuation in an attempt to rebalance folding capacity and demand [3, 4]. In both *Drosophila* cells [5] and mammalian cells [6], toxic accumulation of unfolded proteins may also lead to regulated IRE-1 dependent decay (RIDD) of mRNAs. However, again, this is a general process: instead of degrading only the specific mRNA encoding the toxic protein that triggered the response, many mRNAs encoding secretory proteins are degraded. This is where the current study differs from previously identified mechanisms. By coexpressing wild-type and mutated PPL mRNAs from the same plasmid, the authors found that the presence of wild-type protein and mRNA does not inhibit degradation of mutated mRNAs and, more importantly, the presence of a mutated mRNA does not result in a cellular response that leads to reduction of wild-type mRNA or protein. Thus unlike other pathways, this quality control system, which the

authors term specific mRNA degradation to preemptively regulate aberrant protein production (RAPP), maximizes cellular economy on several levels: mRNA is degraded, ensuring that no continued output of toxic protein is possible, and only the mRNA encoding the mutated protein is degraded, avoiding the energetic costs of a general cellular response.

With the defining features of RAPP in hand, the authors next asked what proteins could be involved in this process. To do so, they adapted elegant biochemical approaches that have previously borne much fruit in identifying components involved in cotranslational secretory protein translocation across membranes [7, 8]. WT and mutated radiolabeled ribosome/nascent chain PPL complexes bearing an N^E-(5-azido-2-nitrobenzoyl)-Lys (ANB-Lys) residue in their signal sequence were synthesized. This photoreactive probe forms adducts with nearby proteins when irradiated with UV light. The authors confirmed that the SRP subunit SRP54 contacts the wild-type PPL nascent peptide. Increasing the number of deleted leucine residues in the PPL signal sequence decreased crosslinking to SRP54 and, simultaneously, a new 100kD photoadduct appeared. This new adduct was determined to contain AGO2 by mass spectrometry, a result that immediately implicates this complex in degrading the mutated mRNA. AGO2 is a known component of the RNA-induced silencing complex (RISC) where it mediates destruction of mRNAs targeted by either microRNAs or siRNAs, and it can also mediate translational repression.

The exciting findings of this paper lead to many new questions, and fleshing out the mechanistic details of this new pathway remains a high priority. Although AGO2 is clearly involved, its precise role is unclear since the authors show that its nuclease activity appears to be dispensable for mRNA degradation. Thus identifying the nuclease involved is a key challenge. Other mRNA degradation pathways involve translational repression: in nonsense-mediated mRNA decay (NMD), for example, the RNA helicase UPF1 in its phosphorylated state contacts the translational initiation factor eIF3 during detection of mRNAs with a premature termination codon, repressing translation prior to destruction [9]. Does the absence of SRP binding to a mutated signal sequence put a similar mechanism into play? Further investigation into RAPP will clearly yield important insights into what is very likely a fundamental mechanism to ensure secretory pathway integrity.

Acknowledgments

Work in the Maquat lab is supported by NIH R01 grants GM059614 and GM074593. M.W.P. is an HHMI Postdoctoral Fellow of the Damon Runyon Cancer Research Foundation, DRG-2119-12.

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Figure 1. Overview of Normal Secretory Protein Biosynthesis and RAPP

Synthesis of wild-type (WT) secretory proteins destined for insertion into the endoplasmic reticulum (ER) proceeds cotranslationally (top).

(a) Secretory proteins encode an N-terminal signal sequence containing a stretch of hydrophobic residues (red) which is encoded by the mRNA template (blue) and emerges from the ribosome during the course of polypeptide elongation.

(b) The signal sequence is recognized by the signal recognition particle (SRP, green) composed of both proteins and RNA, which engages the hydrophobic residues in the signal sequence as well as the ribosome, slowing amide bond formation.

(c) The SRP/ribosome/mRNA/nascent peptide complex is delivered to the ER membrane-embedded SRP receptor, composed of two distinct subunits (purple).

(d) Delivery of the SRP/ribosome/mRNA/nascent peptide complex allows the nascent polypeptide to be inserted into the translocon (green-blue), a proteinaceous channel in the ER membrane that facilitates passage of the polypeptide into the ER lumen. SRP disengages from the nascent peptide and ribosome, polypeptide synthesis resumes, and signal peptidase (brown) cleaves the signal sequence from the polypeptide. Multiple steps along this pathway are powered by GTP hydrolysis.

Regulation of aberrant protein production (RAPP) is a quality control checkpoint for secretory protein synthesis (bottom).

(e) When a mutated N-terminal signal sequence lacking hydrophobic residues (yellow stars) emerges from the ribosome, SRP is unable to engage it.

(f) Instead of SRP, Argonaute 2 (AGO2), which has previously been shown to bind to ribosomal subunits, binds the mutated signal sequence.

(g) AGO2 binding leads to mRNA degradation via yet-to-be-identified nuclease(s) (Pac-man symbols). Degradation could possibly occur via decapping or deadenylation and exo- or endonucleolytic decay (question marks). This ensures that the template encoding the offending polypeptide is destroyed, preventing further rounds of protein synthesis. In contrast to other described quality control pathways operating at the ER, the response (RAPP) to the mutated secretory protein is not global — synthesis of other wild-type secretory proteins continues as normal.