Celsr1-positive vesicular compartments, Fzd6 and Vangl2 are found on distinct vesicular compartments *in vivo*, indicating that antero-posterior core PCP asymmetry is preserved during mitosis. How is this endosomal asymmetry maintained? Devenport *et al.* [8] report that, in cultured keratinocytes, mitotically internalized Celsr1 colocalizes with various endocytic markers, including Rab5- and EEA1-positive early endosomes, Rab11-positive recycling endosomes, as well as caveolin [8]. This observation raises the possibility that anterior and posterior cognate PCP complexes could follow distinct endocytic routes to be targeted to distinct classes of endosomes, thereby preventing the different PCP complexes from mixing in mitosis. During cytokinesis, Celsr1-positive compartments are distributed in a polarized manner at the anterior and posterior poles of daughter cells (Figure 1C). Strikingly, endosomal vesicles are shown to interpret antero-posterior cues independently of mitotic spindle orientation. Stunning mosaic experiments revealed that, at this stage, polarisation of the Celsr1-positive endosomes is dictated in a cell-non-autonomous manner by the interphasic neighboring PCP-polarized cells [8]. Whether and how endosomes containing the anterior or posterior PCP complex selectively recognize and fuse with the respective cognate anterior or posterior cortex remains unknown. It will also be interesting to understand how polarized endosomal recycling drives PCP re-establishment at the boundaries of the two daughter cells (Figure 1D). In addition, future studies will assist our understanding of how PCP complexes from neighboring interphasic cells are maintained at the boundaries of mitotic cells.

What are the underlying molecular mechanisms and the biological relevance of selective mitotic internalization? Using a series of domain swapping and point mutation experiments, Devenport *et al.* [8] reveal that a single juxtamembrane di-leucine signal present in the cytoplasmic domain of Celsr1 is necessary to promote its mitotic internalization. Importantly, in clones of cells expressing the endocytic-defective version of Celsr1, hair follicles are no longer aligned along the antero-posterior axis. Mutant cells align one relative to the other, a misorientation that is transmitted in a dominant cell-non-autonomous manner to adjacent wild-type cells [8]. These observations first strongly argue that mitotic internalization of PCP components is physiologically important and second lead to the proposal that mitotic uptake occurs to prevent PCP signaling from the rounded cell, therefore avoiding disruption of PCP by aberrant directional information. Is this mechanism evolutionarily conserved? Perhaps not, given that mitotic internalization of PCP components has not been reported in *Drosophila* [17,18] and mitotic internalization motif of Celsr1 is not conserved in dipters [8]. How then is PCP transmitted in daughter cells in the fly? Clearly, further investigation of mitotic endocytosis of PCP components in model systems will provide new and exciting insights into how polarized trafficking allows inheritance of PCP in tissues.

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Protein Degradation: BAGging Up the Trash

Cells efficiently uncover and degrade proteins that are misfolded. However, we know very little about what cells do to protect themselves from mislocalized proteins. A new study reveals a novel quality control pathway that recognizes and degrades secretory pathway proteins that have failed to target to the endoplasmic reticulum.

Tslil Ast and Maya Schuldiner*

Have you ever had the dubious pleasure of finding groceries that you've forgotten to place in the refrigerator? Holding your breath and looking away, the only thing left to do is to promptly throw everything out. In

a similar way, cells need to protect themselves from 'spoilt' proteins that have failed to make it to their final cellular destination on time. One large group of proteins that have to be targeted to their cellular destination consists of proteins that either reside in the secretory pathway, are displayed on the plasma membrane or are secreted. Each and every one of these proteins, which usually make up around a third of eukaryotic proteomes, must be targeted in a correct and timely manner to the central portal of the secretory pathway — the endoplasmic reticulum (ER). Once proteins have made it into the ER, its lumen provides a safe folding haven [1]. Although unsuccessful ER insertion is a well-documented phenomenon, there have been no previous studies aimed at uncovering what happens to those proteins that never make it into the ER. Now, in a recent article published in *Nature*, Hessa *et al.* [2] have elucidated a novel mechanism by which secretory proteins that have 'missed their train' and have remained in the cytosol are identified and sent off for degradation.

Translocation into the ER is an intricate, multistep process that involves protein targeting to the ER, gating of the translocon, energy-driven insertion of the protein through the ER membrane, and achievement of the correct orientation of the protein relative to the membrane. Given the complexity of this process, it is not surprising that the efficiencies of ER translocation differ from protein to protein and that a substantial fraction of many ER-bound proteins fail to translocate. Such translocational duds remain in the cytoplasm and constitute a mislocalized protein (MLP) pool [3,4]. Furthermore, even proteins that are efficiently translocated may have difficulties with insertion during times of ER stress, whereby translocational attenuation is induced so as to not overburden the ER with additional folding requirements [5,6].

By their nature, MLPs can interfere with cytoplasmic protein homeostasis. Their exposed hydrophobic domains (signal sequences as well as transmembrane domains not shielded by the membrane) may easily aggregate, and therefore MLPs must rapidly be degraded. Failure to degrade such proteins in an efficient and timely manner can have severe consequences. One notable example is neurodegeneration resulting from the accumulation of the inefficiently translocated prion protein PrP [7]. Furthermore, there are several examples of MLPs contributing to the pathogenicity of Alzheimer's disease [8,9]. While the degradation of MLPs has been shown to involve the proteasome [7], the exact quality control machinery that recognizes and targets these proteins for degradation has remained elusive.

It was previously thought that misfolded proteins in the cytosol would be recognized and degraded by the general cytoplasmic quality control machinery. Indeed, it has been shown that translocation-incompetent ER proteins can be targeted for proteasomal degradation via the cytoplasmic heat shock protein HSP70, which takes part in maintaining general cytoplasmic folding homeostasis [10,11]. However, these studies were carried out on mutated or abnormal proteins, which lacked some of the determinants that are unique to ER proteins — such as transmembrane domains or signal sequences that target the proteins to the ER. It was therefore unclear whether an additional, unique molecular pathway existed to enable recognition and proteasome targeting of MLPs.

Using an *in vitro* system, Hessa *et al.* [2] reconstituted the translation reaction of PrP and the ubiquitination of the cytoplasmic form of PrP, enabling them to elucidate both the factors and the signals that mediate the degradation of MLPs. They elegantly demonstrated that efficient ubiquitination of the cytoplasmic PrP was dependent both on the release of the protein from the ribosome (without having yet been inserted into the ER membrane) and on the presence of long linear hydrophobic stretches of the protein. Specifically, PrP contains two such hydrophobic domains — an amino-terminal signal sequence, which targets it for ER insertion, as well as a carboxy-terminal sequence, which facilitates the attachment of a glycosyl phosphatidylinositol (GPI) anchor. Deletion of both of these sequences from PrP markedly reduced its ubiquitination, while the addition of these sequences or transmembrane domains to GFP induced its ubiquitination. These findings indicated that it is not merely the unfolded nature of the MLPs that targets them for degradation, but rather the presence of sequences that are unique to proteins that must enter the secretory pathway.

By carrying out crosslinking, fractionation and reconstitution assays, Hessa *et al.* [2] were able to identify the machinery that carries out the ubiquitination of cytoplasmic PrP. Strikingly, they discovered that MLPs that have been released from the ribosome are bound by BAG6, which maintains them in a ubiquitination-competent state (Figure 1). BAG6, also known as BAT3 or Scythe, has been identified to take part in the post-translational insertion of tail-anchored (TA) proteins into the ER [12,13] (Figure 1). Together with TRC35 and UBL4A, BAG6 makes up a tri-chaperone ribosome-associated complex, which binds TA proteins post-translationally, and loads them onto the cytosolic ATPase TRC40, which targets these proteins to the ER [12,14–16]. However, in the case of MLPs, BAG6 does not proceed to hand off the proteins to TRC40, but rather recruits the ubiquitination machinery through its amino-terminal UBL domain. Hessa *et al.* [2] demonstrated that the E2 ubiquitin-conjugating enzyme that takes part in the ubiquitination reaction of MLPs is UBCH5. However, the E3 ubiquitin ligase that participates in this ubiquitination remains so far unknown (Figure 1).

This mechanism of MLP identification and ubiquitination that was initially uncovered in the *in vitro* systems seems to hold true *in vivo*. Specifically, the cytoplasmic form of PrP was stabilized in cultured cells that overexpressed a version of BAG6 that lacks its UBL domain. This degradation was dependent on the presence of hydrophobic stretches on PrP, such as the signal sequence and the GPI attachment sequence. Moreover, these *in vivo* experiments showed that BAG6 is a real multitasker — even tagging TA proteins for degradation in the absence of TRC40 (Figure 1).

More generally, these findings together with previous work [12,16] suggest that BAG6 can bind terminal hydrophobic stretches (the carboxyl terminus in the case of TA proteins or, in the case of signal-sequence-containing proteins, the GPI attachment sequence or the amino terminus) and act as

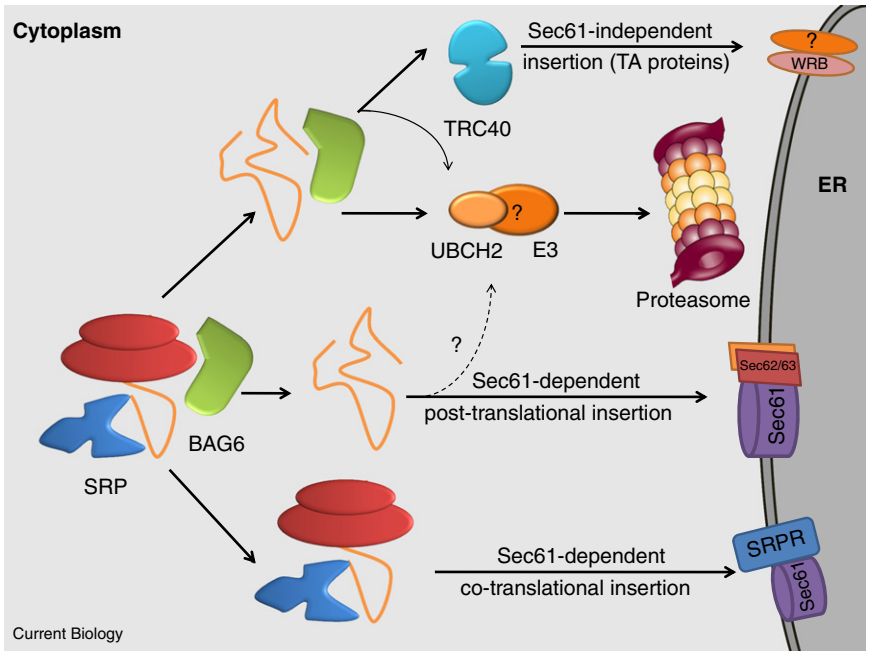

Figure 1. A schematic representation of the triage functions of BAG6 in protein translocation and degradation.

Secretory pathway proteins are thought to enter the endoplasmic reticulum (ER) through three routes: co- or post-translational translocation dependent on Sec61 (translocon) and Sec61-independent post-translational translocation. Hessa *et al.* [2] show that translating ribosomes bind both the signal recognition particle (SRP) and BAG6. If the signal sequence is recognized by SRP, translational halt and co-translational translocation will occur. In the absence of SRP binding, two types of protein bind BAG6 upon ribosomal release: first, tail-anchored (TA) proteins require BAG6 to be loaded onto TRC40 for Sec61-independent post-translational translocation. Second, secretory pathway proteins that have failed to insert into the ER will bind BAG6. In this scenario, BAG6 recruits the ubiquitination machinery, thereby targeting these mislocalized proteins for degradation. Some major open questions remain, such as whether Sec61-dependent post-translational translocating proteins that do not bind SRP utilize BAG6 either for insertion or for quality control, what is the identity of the E3 ubiquitin ligase that ubiquitinates BAG6 substrates, and how is the ratio of ER-inserted versus degraded protein determined for each gene.

a triage factor. TA proteins are passed on to TRC40 for ER insertion if possible and targeted for degradation when not, while MLPs are always tagged for degradation. This dedicated pathway appears to couple translation and degradation. It ensures the rapid capture of any MLPs that have failed to translocate, thereby precluding their aggregation in the cytosol as well as the futile recruitment of cytoplasmic folding machinery. Moreover, BAG6 may have additional roles in maintaining the folding homeostasis of the cytoplasm, as it has been shown to mediate the degradation of defective ribosomal products [17], which make up about a third of newly synthesized polypeptides [18].

Hessa *et al.*'s [2] work has answered a fundamental cell biological question regarding MLP degradation. Like every good new insight into cell biology, it now begs further queries, regarding both the determinants and the mechanisms that drive degradation. For example, co-translationally targeted proteins bind the signal recognition particle (SRP) as they emerge from the ribosome. How then is the fraction of targeted (SRP-bound) versus degraded (BAG6-bound) proteins determined for any given gene? This seems to be a regulated and dynamic ratio, indicating that there are *trans*-factors that modulate this balance. In addition, how much time is a protein given to be inserted into the ER before it is targeted for BAG6-dependent degradation? Finally, how is the handoff between BAG6 and the degradation machinery carried out? BAG6 has been shown to bind to poly-ubiquitinated proteins [17] — could this triage protein also chaperone MLPs to the proteasome?

Together, these findings have opened an exciting and new field of pre-insertional quality control. It seems that life on the secretory export track is rough — upon exiting the ribosomes, polypeptides have to hurry and bind the SRP or be translocated, otherwise a clearance mechanism kicks in, rapidly BAGging up the trash, and ensuring that the cytosol is kept clean and tidy (Figure 1).

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Affective Neuroscience: Tracing the Trace of Fear

The trace of fear has been elusive and difficult to discern in the human brain. Researchers have come up with a clever new way to track it down.

Daniela Schiller

"*Even though I walk through the valley of the shadow of death, I will fear no evil*" says David in the 23rd Psalm. Had we scanned David's brain while walking through the dark valley of death, what would we see? Could we track his fears surface and crumble? Bach *et al.* [1] would say, yes; last month they reported in *The Journal of Neuroscience* a novel way to identify a stable but sparse fear memory trace in the human amygdala.

Tracing the trace of fear in the human brain is not an easy task. Researchers of the human brain have limited access. They cannot insert electrodes, cut slices, or inject toxic drugs, unless a medical condition calls for it. Most of what we know about the human brain, we derive from animal studies. From the middle of the 20th century until this very day [2,3], the conclusions from animal research are loud and clear — the amygdala is critical for the acquisition and expression of fear. Draw an imaginary line passing through your ear into the brain, and then another line through your eye: the amygdala roughly resides where these two lines meet (Figure 1). The amygdala has a mechanism to detect and predict threats; this mechanism is so highly conserved in evolution that we spot it essentially everywhere, in a rat and a human being alike.

The amygdala is in fact a conglomerate of sub-nuclei, rather than a cohesive brain region (Figure 1). Although falling under the same corporate structure, each of the sub-nuclei engages in a completely different business. The lateral nucleus, for example, is where inputs into the amygdala converge. The central nucleus is the output station. In between lies an island of inhibitory neurons, the intercalated cells, which transmit information within the amygdala, and so forth [4–6]. Invasive techniques, such as electrophysiological recording, allow such detailed investigation. In humans, instead, we use a non-invasive method called functional magnetic resonance imaging (fMRI).

Imaging by this method divides the brain volume into spatial units called voxels, which are analogous to pixels but in a three-dimensional space. Voxel sizes typically range between one to three cubic millimeters. Each voxel therefore contains millions of neurons and it does not map onto any naturally occurring layout of the brain. The collective activation of those millions of neurons sums up into one data point. The fMRI method does not, in fact, register direct neuronal firing in each data point; it actually reflects the impact of the collective neural activity on nearby blood vessels. To make things even blurrier, each brain region contains hundreds to thousands of voxels. The amygdala, for example, hosts about 2000 voxels in each side of the brain. Because of issues of noise and statistical power, fMRI studies usually report the average activation from all these voxels, or at least, from the few most active ones. Studies using fMRI also conventionally perform group analyses and seldom examine individual participants.

We are facing a double-edged sword then. On the one hand, fMRI gives us safe access into the human brain, producing beautiful maps of the evolving activation from every corner of the brain simultaneously. On the other hand, it does so with poor resolution. Ten years ago, Haxby *et al.* [7] came up with a clever new way to deal with these data. Instead of lumping neighboring voxels together, they inspected multiple voxels in parallel, to see what kind of pattern they create. Think of a group of sixth grade kids. According to the traditional approach, you would take the height of each kid, and end up with a number representing the class's average height. Following Haxby's approach, you would ask all kids to stay where are sitting and give each one a flashlight that beams relatively to their height: bright yellow to the tallest, turning orange-red the shorter they are. You would then look at the colorful shiny pattern they create when they all turn on their flashlights at the same time.

Haxby *et al.* [7] showed how each multi-voxel pattern of activity is in fact a marker of a particular cognitive state. When their participants viewed different categories of images (faces, houses, furniture, and so on), each category produced a distinct pattern in the visual cortex. What could we do with such a technique? Mind reading. Not to invade the privacy of your thoughts, but rather as a genuinely useful tool to understand how the brain processes information. If we decode a pattern reflecting an arm movement in the brain of a hemiplegic, for example, we could feed it into a robotic arm to perform the action that the hemiplegic desires.

But Bach *et al.* [1] had other intensions. They ingeniously proposed