pH, mutation, or chaotropes, then triggers *cis*/*trans* isomerization of the His3-Pro32 peptide bond and conversion of the protein to the amyloidogenic state. Unraveling the subsequent structural evolution of the protein during later stages of amyloid formation awaits further experimentation, although progress is being made (for example, see Debelouchina et al., 2010). It will be exciting to see how the population of a rare conformer that has a strong tendency for intermolecular association then leads to the regular cross-β structure that is a hallmark of amyloid fibrils.

How do these results explain amyloidogenicity of β_2m shed from cells? Presumably, environmental factors within the body trigger conversion to an amyloidogenic state by tickling the N-terminal region of this metastable protein. Following this logic, proteolytic cleavage of the N-terminal portion of plasma β_2m would not only lead to increased amyloidogenicity of the clipped product but also create a species that can cause the conversion of fellow full-length soluble β_2m species into amyloidogenic states (Figure 1D). How, where, and when the cleavage of β_2m to produce ΔN6 occurs physiologically must now be answered. If this cleavage is a major factor in DRA, then inhibition of β_2m proteolysis emerges as a potential route toward therapy.

Why does evolution allow the risk of β_2m amyloidosis to continue? There are two intriguing considerations: First, the metastability of this protein may confer an advantage at an earlier stage, here most likely in the formation of MHC-I complexes. This may be due to the timing of *trans-cis* isomerization, for example, which may help coordinate folding of a multichain complex. This would be similar to the folding of immunoglobulins, which is also rate-limited by peptide bond isomerization of the light chains (Feige et al., 2009). The evolutionary selection for a metastable native state is reminiscent of serpins. They too are vulnerable to aggregation-associated pathologies, but the risk is acceptable to preserve their capacity to perform wide-ranging and essential functions (Whisstock and Bottomley, 2006). Second, the risk of DRA from β_2m arises only during kidney failure and thus is part of a more complex set of physiological breakdowns. It is difficult to imagine an evolutionary route to minimizing secondary pathological consequences in the presence of a primary organ failure.

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Regulator of Ras Depalmitoylation and Retrograde Trafficking: A New Hat for FKBP

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In this issue of *Molecular Cell*, Ahearn et al. (2011) identified FKBP12 as a novel regulator of Ras signaling through its modulation of depalmitoylation of H-Ras and its recycling from plasma membrane to the Golgi.

FKBP's name tells a lot about its history but preciously little about its true cellular functions. FKBP stands for FK506-binding protein, as it was initially identified as a high-affinity binding protein and a putative target for the immunosuppressive drug FK506 (Harding et al., 1989; Siekierka et al., 1989). As a relatively large family of abundant cytosolic proteins that is conserved from bacteria to humans, of course, FKBP was not there to help a bacterial natural product FK506 to block

calcium signaling and the human immune response in the first place. The first clue on the physiological function of FKBP came when FKBP was found to possess peptidyl-prolyl *cis-trans* isomerase (or prolyl isomerase) activity that catalyzes the rate-limiting step of the conformational change of proline-containing peptide bonds during protein folding (Fischer et al., 1984; Harding et al., 1989; Siekierka et al., 1989). For quite some time, the hypothesis prevailed that the prolyl isomerase activity of FKBP may play an essential role in intracellular calcium signaling. But the hypothesis was refuted with the discovery of calcineurin as the ultimate target for FK506, through binding of the FKBP-FK506 complex to calcineurin, leaving the physiological functions of FKPB as a mystery (Liu et al., 1991). That mystery reached a climax when all four known isoforms of FKBP along with eight isoforms of another family of prolyl isomerase cyclophilin were knocked out simultaneously in the budding yeast and no obvious phenotype was discernable in the resulting knockout mutant (Dolinski et al., 1997). Over the ensuing years, however, clues as to the functions of FKBP in higher eukaryotes began to trickle in, and FKBP gradually turned from a functionally "bald" protein into one with more and more "hats." In the latest episode, Ahearn et al. uncovered another unexpected hat for FKBP12 (the 12 kD isoform of FKBP)—a regulator of the depalmitoylation of Ras and its retrograde trafficking from the plasma membrane to the Golgi (Ahearn et al., 2011).

Ras proteins are small GTPases that play important roles in transmitting signals from growth factors to regulate cell growth and proliferation. They gained notoriety as mutations in Ras were frequently found in human cancer cells and oncogenic Ras was shown to be one of the ubiquitous drivers of human cancer. The four subtypes of Ras isoforms, H-Ras, N-Ras, K-Ras4A, and K-Ras4B, share a common type of C-terminal lipid modification—attachment of a farnesyl group to the C-terminal cysteine residue that enables them to be associated with the plasma membrane in close proximity to their upstream activators. In addition to farnesylation, H-Ras and N-Ras undergo additional palmitoylation at cysteine residues close to their C termini. For a long time, it was thought that the dual lipid modifications of H- and N-Ras were to enhance their association with membranes. Some 5 years ago, two groups uncovered an important new role of palmitoylation (Figure 1). It turned out that H- and N-Ras undergo dynamic cycles of palmitoylation/depalmitoylation, which are accompanied by changes in their subcellular localization between the plasma membrane and the Golgi/ER (Goodwin et al., 2005; Rocks et al., 2005). Palmitoylated Ras tends to accumulate in the plasma membrane while depalmitoylated Ras gets recycled to the Golgi where it is palmitoylated again and transported via vesicles back onto the plasma membrane. This finding not only shed significant new light on the spatiotemporal regulation of signaling by Ras, but also raised a new question, i.e., what regulates the palmitoylation and depalmitoylation cycle of Ras?

In attempts to further deconvolute the mechanism regulating the recycling of H-Ras between the plasma membrane and the Golgi, Ahearn et al. (2011) fused H-Ras and its C-terminal 10 amino acid tail to mCherry and GFP, respectively, and compared their distribution between the plasma membrane and the Golgi. It was found that the 10 amino acid C-terminal tail of H-Ras failed to show up in the Golgi. In contrast, expansion of the C-terminal tail from 10 to 19 amino acids recapitulated the distribution of full-length H-Ras, suggesting that the additional 9 amino acid sequence adjacent to the C terminus played a key role in enabling plasma membrane-Golgi recycling of H-Ras. Inspection of the 9 amino acid sequence revealed that it is rich in proline, leading to the hypothesis that peptidyl prolyl *cis-trans* isomerization and the enzyme that catalyzes the reaction, FKBP12, may be involved in regulating the subcellular distribution of H-Ras and its palmitoylation/depalmitoylation. In a series of experiments, the authors convincingly showed that FKBP12 indeed facilitated the depalmitoylation of H-Ras (Figure 1). Inhibition of FKBP12, but not FKBP38, using either small molecule inhibitors including FK506 and rapamycin or RNA knockdown, prevented depalmitoylation of H-Ras and confined it to the plasma membrane. FKBP12 was shown to specifically bind palmitoylated Ras and promote its depalmitoylation in a manner that is dependent on the presence of P179 in H-Ras and the prolyl isomerase activity of FKBP12. Moreover, inhibition of FKBP by FK506 was shown to enhance Ras signaling in the cellular context of Ras-mediated neurite outgrowth in PC12 cells, providing a plausible mechanism to the long-standing question of how FKBP ligands stimulate the growth of neurites (Lyons et al., 1994).

The crosstalk between FKBP12 and Ras unraveled in this work adds yet another level of regulation of Ras activity through a new type of posttranslational modification, i.e., protein conformational change. The distinct types of posttranslational modifications of Ras including farnesylation and palmitoylation serve to generate multiple states of the protein with distinct activities. Thus, farnesylation

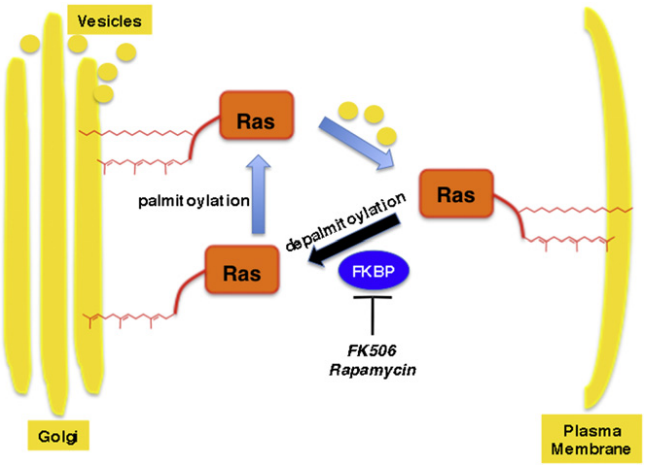

Figure 1. Regulation of Ras Depalmitoylation and Recycling from the Plasma Membrane to the Golgi by FKBP
Ras includes H- and N-Ras and FKBP refers to FKBP12. For simplicity, only one palmitoyl group is symbolically shown at the C-terminal tail of Ras.

is responsible for anchoring Ras to membranes. Palmitoylation seems to divide H- and N-Ras into two spatially separate pools, one on the plasma membrane and the other on the Golgi. To interconvert between the palmitoylated and depalmitoylated states, a proline residue, P179, appears to be a new switch. The presence of P179 gives rise to two distinct, *cis* and *trans*, conformations of Ras around the P179-containing peptide bond. It is possible that only one of two conformations of Ras is conducive to the depalmitoylation reaction, which necessitates the involvement of FKBP12 to catalyze the interconversion of the two conformational states of Ras.

Like most other enzymes, the association of FKBP12 and its substrates has been shown to be transient. That FKBP forms a stable complex with palmitoylated Ras while catalyzing the *cis-trans* P175 peptide bond isomerization is new and unprecedented. It has been shown that Pin1, a member of the parvulin family of prolyl isomerase, forms stable complexes with its substrates (Finn and Lu, 2008). But Pin1 does so through an additional WW domain that mediates its interaction with most, if not all, of its substrates. In contrast to Pin1, FKBP12 is a relatively small, compact, and single-domain protein with one active site. How this relatively small active site accommodates both a G-P dipeptide moiety and the adjacent palmitoyl Cys residues in Ras remains to be elucidated at the structural level. Furthermore, how FKBP12 regulates depalmitoylation through its stable association with Ras and its prolyl isomerase activity in conjunction with the elusive thioesterase that depalmitoylate Ras poses yet another puzzle. Last but not least, the findings disclosed in this work raised important questions about the potential effects of the widely used drugs FK506 and rapamycin on cancer. On one hand, as inhibition of FKBP was shown to enhance Ras activity on the plasma membrane, could FK506 and rapamycin promote tumor formation and growth? On the other hand, given the dependence of Ras signaling on its palmitoylation/depalmitoylation and the accompanying plasma membrane/Golgi cycles, could FK506 and rapamycin possess previously unappreciated antitumor activity? For now, only time and more vigorous experiments will tell what may come out of the new magic hat of FKBP12 as a regulator of H- and N-Ras depalmityoylation.

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Many Paths to the Same End: Histone Transcripts Recruit Canonical Initiation Factors through Unconventional Interactions

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In this issue of *Molecular Cell*, Eriani and colleagues show that histone transcripts recruit translation initiation factors through an alternate pathway reminiscent of viral translation, suggesting a broader role for noncanonical translation initiation on cellular transcripts (Martin et al., 2011).

In the standard model of eukaryotic translation initiation, factors are recruited to a distinctive m^7GpppG cap structure present at the 5′ end of transcripts, where they assemble into a complex that scans down the message and begins translation at the first AUG codon it encounters (Sonenberg and Hinnebusch, 2009). The cap is bound directly by the initiation factor eIF4E, which in turn interacts with the scaffold eIF4G. Interestingly, eIF4G also associates with poly(A) binding