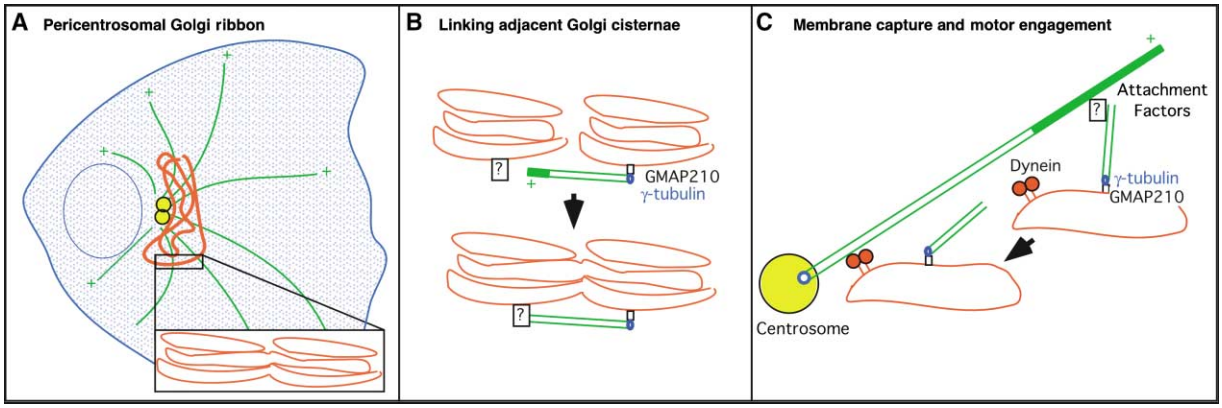

Figure 1. Speculative Models of GMAP-210 Function in Linking and Positioning Golgi Membranes

(A) The mammalian Golgi forms a pericentrosomal ribbon-like membrane system comprised of linked ministacks.

(B) Golgi-localized GMAP-210 may bind γ-tubulin complexes leading to the presence of short, stabilized microtubules on the Golgi ministacks. These could be linked to adjacent ministacks by other Golgi-localized microtubule binding proteins (e.g., CLASP2, which binds and stabilizes microtubule plus ends).

(C) Alternatively, GMAP-210 bound Golgi microtubules may facilitate capture of Golgi membranes by outgrowing centrosomal microtubule plus ends and the subsequent engagement of dynein/dynactin complexes. Attachment factors may include CLASP2, CLIP-59, BICD2, Hook3, $p150^{Glued}$, golgin-84, TMF/ARA160, or others.

engagement (Figure 1C). Indeed, a critical next step is to determine whether Golgi motility is impaired in cells after GMAP-210 knockdown. Molecular requirements for motility may be much more complex than previously imagined. Evidence suggests that Golgi membranes must encounter and be loaded onto growing microtubule plus ends prior to inward motility (Vaughan et al., 2002). This reaction is reminiscent of kinetochore search and capture carried out by microtubules during mitosis. It may be that many of the Golgi cytoskeleton "linking" proteins mediate pericentrosomal ribbon formation by allowing centrosomal microtubule plus ends to attach to Golgi membranes and to engage the dynein-dynactin motor complex. Further research is clearly necessary.

Although it is tempting to speculate that trafficking to the Golgi, and processing in the Golgi, is more efficient in a single central organelle, there is no clear evidence that this is the case. On the other hand, efficiency of post-Golgi transport in large polarized cells profits from centrosomally organized microtubules. Thus, pericentrosomal positioning of the Golgi apparatus could be a byproduct of a strategy using microtubules and plus end-directed motility to orient delivery of post-Golgi membrane carriers over large distances and to specific cellular sites. Interestingly, with gene products such as GMAP-210 in place to maintain a central Golgi ribbon, Golgi positioning itself may have evolved additional roles. A dramatic example is provided during cell division when Golgi membranes normally vesiculate and disperse. Failure of Golgi dispersal is associated with severe mitotic defects, indicating that Golgi fragmentation is a necessary part of cell cycle progression (Colanzi et al., 2003). In this light, the mechanisms now being elucidated for positioning of the Golgi apparatus have significance at many levels.

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Myosin Assembly: The Power of Multiubiquitylation

Ubiquitylation provides a means of targeting substrate proteins for degradation by the proteasome. Novel findings in *C. elegans* (Hoppe et al., 2004, this issue of *Cell*) establish that two ubiquitin-ligases team up to multiubiquitylate the myosin chaperone UNC-45, suggesting a novel link between regulated protein degradation and myosin assembly.

Selective protein degradation by the 26S proteasome plays a crucial role in the homeostasis of eukaryotic cells. The proteasome participates both in the regulated proteolysis of functionally active proteins and in the degradation of aberrantly folded polypeptides. Substrates

destined for proteasome-mediated degradation are marked by the covalent attachment of ubiquitin, through a sequence that involves an E1 ubiquitin-activating enzyme, an E2 ubiquitin-conjugating enzyme, and an E3 ubiquitin-ligase (Pickart, 2004). This sequence can be iterated and multiubiquitylated proteins are preferred substrates of the proteasome. In some cases, efficient multiubiquitylation requires a distinct ubiquitylation factor termed E4. *S. cerevisiae* UFD2, the first E4 to be described, recognizes substrates bearing one to three ubiquitin molecules and catalyzes addition of further ubiquitin moieties in the presence of E1, E2, and E3 enzymes, thus yielding multiubiquitylated substrates (Koegl et al., 1999). UFD2 defines a protein family that bears a U-box thought to be structurally related to the RING finger found in many E3 ubiquitin-ligases (Aravind and Koonin, 2000). Human CHIP (carboxyl-terminus of Hsc70 interacting protein) is another U-box-containing protein that can act as an E4. CHIP promotes multiubiquitylation of the unfolded membrane protein Pael-R by the E3 enzyme Parkin, a protein affected in familial Parkinson's disease (Imai et al., 2002). This finding notwithstanding, the nature of in vivo substrates for CHIP or other E4 remained poorly characterized. Exciting findings by Hoppe and colleagues published in this issue of *Cell* establish that UFD-2 and CHN-1, the *C. elegans* homologs of UFD2 and CHIP, act in concert to multiubiquitylate the myosin chaperone UNC-45, suggesting that selective protein degradation contributes to proper myosin assembly (Hoppe et al., 2004).

The authors first characterized the biochemical activity of UFD-2, as well as that of CHN-1, which they identified in a yeast two-hybrid assay as a binding partner of UFD-2. In the presence of appropriate E1 and E2 enzymes, both UFD-2 and CHN-1 underwent self-ubiquitylation, as is characteristic of ubiquitin-ligases. Next, the authors made the key finding that UFD-2 and CHN-1 both interact with purified UNC-45 in vitro. UNC-45 is a member of the UCS (UNC-45/CRO1/She4p) family of proteins that are required for myosin assembly (Hutagalung et al., 2002). UNC-45 helps ensure that muscle myosin is properly folded through a dual mechanism: by exerting chaperone activity directly on the myosin head and by acting as a cochaperone for the heat-shock protein Hsp90 (Barral et al., 2002). As might be expected from such a role, *unc-45* mutant animals exhibit severely disorganized myofibrils and dramatically decreased body movements. What is the consequence of the physical interactions detected between UNC-45 and UFD-2 or CHN-1? Importantly, Hoppe and collaborators (2004) found that UNC-45 is a substrate for UFD-2- and CHN-1-dependent ubiquitylation. When either UFD-2 or CHN-1 was present alone, most of the UNC-45 conjugates contained only one to three ubiquitin moieties, whereas simultaneous addition of UFD-2 and CHN-1 dramatically enhanced multiubiquitylation of UNC-45. These findings indicate that *C. elegans* UFD-2 and CHN-1 merely act as E3 ubiquitin-ligases on their own and suggest a novel mechanism in which E4 activity is achieved by formation of a heterooligomeric complex comprised of two E3 enzymes.

What is the in vivo relevance of the interactions observed in vitro? Hoppe and colleagues (2004) analyzed the distribution of GFP driven by the *ufd-2* or the *chn-1* promoter. The fusion proteins displayed an overlapping expression pattern that included body wall muscle cells, which also express UNC-45. Therefore, UFD-2, CHN-1, and UNC-45 are all present at the crime scene. Moreover, a functional FLAG-tagged UNC-45 was ubiquitylated in worm lysates, compatible with the in vitro activities of UFD-2 and CHN-1. The authors isolated a deletion allele of *chn-1*, which displayed no obvious morphological defects under standard growth conditions. Importantly, however, several phenotypic manifestations caused by a temperature-sensitive allele of *unc-45* were significantly suppressed by the absence of *chn-1*. In contrast, analogous phenotypes caused by mutations in other body wall muscle components were not suppressed, indicating specificity of the genetic interaction between *unc-45* and *chn-1*. Whether a similar interaction exists between *unc-45* and *ufd-2*, and whether the combined absence of *ufd-2* and *chn-1* would potentiate these effects, awaits isolation of an *ufd-2* mutant allele. In a converse set of experiments, the authors established that overexpression of UNC-45 in body wall muscle cells, while being of no consequence in a wild-type background, resulted in dramatic muscle defects in the *chn-1* mutant background. Together, these findings indicate that CHN-1 is a negative regulator of UNC-45 in muscle cells, most likely via its ability to promote multiubiquitylation in concert with UFD-2. Whereas the biological function of this modification remains to be determined experimentally, an appealing hypothesis is that UNC-45 multiubiquitylation promotes its degradation by the proteasome. In this view, multiubiquitylation is crucial for proper myosin assembly by setting the levels of UNC-45 available for aiding myosin folding.

In the future, it will be interesting to elucidate the mechanism by which UFD-2/CHN-1 ensures multiubiquitylation of UNC-45. Perhaps complex formation stabilizes conformations of UFD-2 and CHN-1 that allow optimal ubiquitin ligation. Alternatively, such a complex may be necessary for ubiquitin-ubiquitin linkages that engage different lysine residues on the ubiquitin polypeptide than those utilized for monoubiquitinylation, as has been suggested in the case of *S. cerevisae* UFD2 (Koegl et al., 1999). Regardless of the mechanism, the findings of Hoppe and colleagues (2004) reveal an unsuspected plasticity among E4 enzymes. Whereas *S. cerevisiae* UFD2 and human CHIP can each individually exert E4 activity, formation of an UFD-2/CHN-1 heterooligomer is essential for UNC-45 multiubiquitylation in *C. elegans*. Interestingly, homodimerization is needed for CHIP activity (Nikolay et al., 2004), raising the possibility that a CHIP homooligomer functions in an analogous manner to a UFD-2/CHN-1 heterooligomer. Hoppe and collaborators (2004) speculate that formation of such heterooligomers may offer added plasticity for metazoan organisms, because degradation of specific substrates could be induced by developmentally regulated coexpression of a combination of appropriate E3 enzymes.

A careful balance between molecular chaperones, which help fold proteins, and enzymes of the ubiquitylation pathway, which promote their degradation, ensures adequate protein homeostasis in eukaryotic cells. CHIP is central to this balance in neuronal cells, not only because it enhances the ability of Parkin to multiubiqui-

tylate Pael-R, but also because it leads to dissociation of Hsp70 from Parkin and Pael-R, thus further driving Pael-R degradation (Imai et al., 2002). It will be interesting to determine whether the UFD-2/CHN-1 complex may similarly lead to dissociation of Hsp90 from UNC-45, something that could contribute to rendering it more prone to degradation. UNC-45 proteins are conserved across metazoan evolution (Hutagalung et al., 2002) and UFD2, as well as CHIP, are abundantly expressed in human muscle cells (Ballinger et al., 1999; Mahoney et al., 2002). Therefore, the regulation of myosin assembly via multiubiquitylation of UNC-45 uncovered in *C. elegans* should have important implication for understanding myosin assembly during physiological and pathological conditions in humans. Moreover, since other UCS proteins interact with unconventional myosin, increased understanding of the mechanisms by which multiubiquitylation regulates their function will undoubtedly shed light on other myosin-dependent processes such as cell motility and cytokinesis.

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Gene Targeting: Attention to Detail

When it comes to silencing genes in mice, not all approaches are equal. An example published in this issue of *Cell* (Patrucco et al., 2004) suggests that caution should be used when validating potential drug targets by genetic disruption

Gene targeting in mice is a powerful tool for uncovering the physiological roles of proteins and is increasingly being used for preclinical validation of potential new drug targets (Harris, 2001). Different experimental strategies of gene targeting can, however, give rise to very distinct phenotypes. The commonest pitfalls include "neighborhood" phenotypic effects caused by retention of selection cassettes in the targeted locus, multiple gene inactivation, and creation of truncated biologically active gene products, any of which can lead to "off-target" phenotypes. It is also becoming increasingly clear that "knocking out" (KO) a gene is not the equivalent of "knocking in" (KI) an inactivating point mutation or an otherwise disabled full-length version of the gene. A prime example highlighting how different gene inactivation strategies can lead to strikingly different phenotypes is published in this issue of *Cell* (Patrucco et al., 2004). In this study, Hirsch's group inactivated p110γ, a member of the phosphoinositide 3-kinase (PI3K) gene family, by knocking in a kinase-dead version into the endogenous p110γ gene locus, and observed a very unexpected result.

PI3Ks generate lipid signals downstream of receptors and are considered to be good targets for pharmacological intervention in diseases associated with deregulated PI3K activity, such as cancer, inflammation, and diabetes. There are several isoforms of PI3K linked to different receptor systems and with apparently discrete functions in cells and in the organism (reviewed in Vanhaesebroeck et al., 2001). These include the p110α, β, and δ isoforms that play a role in tyrosine-kinase-signaling pathways. In contrast, p110γ transmits PI3K signals downstream of G protein-coupled receptors (GPCRs). p110γ is highly expressed in white blood cells and the phenotype of the p110γ KO mouse, published by three independent groups in *Science* a few years ago (Dekker and Segal, 2000), seemed straightforward at first. These p110γ KO mice were apparently healthy but had a dampened immune response when challenged with inflammatory stimuli. This phenotype was due to decreased inflammatory migration of macrophages and reduced production of inflammatory substances by neutrophils. These studies caused quite a stir and put p110γ on the top of the wish list of many pharmaceutical companies in their quest for new anti-inflammatory drug targets.

This enthusiasm was greatly tempered by a follow-up report that the p110γ KO mice developed colon cancer (Sasaki et al., 2000). Given that inflammation often requires long-term treatment with drugs, inducing cancer would certainly be an undesirable side effect of p110γ inhibitors. However, independently created p110γ KO mouse lines did not develop cancer (Barbier et al., 2001). The exact reason for the isolated case of a cancer phenotype is still not clear (Sasaki et al., 2003) but is possibly related to mutations acquired in the ES cells during culture and gene targeting. This finding put the minds of p110γ drug developers at rest, but only for a little while.

Further alarm bells went off when it became clear that the p110γ KO mice, apart from an immunological phenotype, also had a cardiac defect, namely increased contractility (Crackower et al., 2002). PI3K action has been implicated in cardiac function and several GPCR-signaling pathways, including those triggered by adrenergic receptors, have important functions in the heart