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aMAGEing New Players Enter the RING to Promote Ubiquitylation

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The MAGE proteins are best known as curious tumor-specific antigens. However, Doyle et al. (2010) reveal that MAGE proteins interact with RING proteins to promote ubiquitylation which provides important new insights into the physiological and pathological functions of this enigmatic family of proteins.

The MAGEs have been in the limelight for years as tumor-specific antigens, but in this issue of *Molecular Cell*, Doyle et al. (2010) cast a whole new light on this cryptic family of proteins, revealing their interactions with RING proteins as a unifying theme that may be at the heart of both their normal and pathological functions. The first human melanoma antigen (MAGE) gene, MAGE-A1, came on the scene in 1991, when it was identified as the precursor of a tumor antigen specifically presented on melanoma (van der Bruggen et al., 1991). In the intervening years, 60 MAGE genes have been identified, which share a central and conserved 165–171 amino acid module, the mysterious MAGE homology domain (MHD) (Chomez et al., 2001). The MAGEs are subdivided into two categories based on their chromosomal location and expression. MAGE II genes (15 total) are widely expressed and have roles in cell cycle withdrawal, neuronal differentiation, and apoptosis (Barker and Salehi, 2002). The MAGE I genes (45 total), located in clusters on the X chromosome, are normally restricted in their expression to the testis, trophoblast, and placenta (Chomez et al., 2001). MAGE I gene expression is silenced during development by promoter DNA methylation. However, during the epigenetic reprogramming that occurs in many tumors, MAGE I promoters become hypomethylated, triggering their aberrant expression and presentation as tumor-specific antigens (Simpson et al., 2005).

A burgeoning focus has been on the potential of MAGE proteins as pre-emptive anticancer vaccines, which has overshadowed more fundamental questions, including whether the MHD module shared by both MAGE I and II genes has a common structure and function that is important in normal physiology and cancer. Doyle et al. now approach this question by identifying the cellular interacting partners of six MAGE I proteins and three MAGE II proteins in a human embryonic kidney cell line (293 cells) and demonstrate that the MHD is composed of two winged-helix motifs (Figure 1A). They reveal that a unifying theme is the interaction of the conserved MHD domain in MAGE I and II proteins with cellular proteins that have a really interesting new gene (RING) domain. However, in a surprising twist, they show that the MHD domains of MAGE proteins bind not to the RING domain but instead to disparate and apparently unrelated protein modules in their RING interaction partners. For example, the MHD of MAGE-G1 interacts with the double winged-helix domain of NSE1, the MHD of MAGE-B18 with a basic region in LNX1, and the MHD of MAGE-C2 with the coiled-coil domain of TRIM28 (Figure 1). Thus, unlike other conserved protein modules (Pawson and Nash, 2003), the MHD domain appears to be very flexible and does not converge on proteins with a common motif but, rather, a common function. The authors go one step further and solve the crystal structure of the MAGE-G1-NSE1 complex, confirming that the RING domain is not involved and that the interaction is between the respective MHD and NSE1 winged helices. Their structure of the MAGE-G1 MHD is similar to that of the recently solved MAGE-A4 MHD (PDB: 2WA0). However, one striking difference is the relative orientations of the winged helices, suggesting that the MAGE-G1 MHD undergoes a conformational change upon binding to NSE1. A major question is whether the interaction of the MHD with NSE1 can be extrapolated to other MAGE-RING interactions or is instead a singular example, perhaps reflecting the ability of winged helices to dimerize. It is fascinating to consider how such functional specificity and binding plasticity coevolved in such a conserved domain. A key clue may be the variable length and composition of the disordered linker between the winged helices, which may facilitate interactions with disparate protein domains or arrange the MHD "wings" in many different binding configurations. Exciting answers await us when the structures of other MHD-RING-interacting complexes are solved.

Putting the structural questions aside, the authors address the functional and biological relevance of MHD-RING protein complexes. In most proteins, the RING domain is associated with E3 ubiquitin ligase activity (Deshaies and Joazeiro, 2009). Using in vitro assays, the authors show that MAGE-G1 and MAGE-C2 enhance the ubiquitin ligase activity of NSE1 and TRIM28, respectively. TRIM28 has been shown to target the p53 tumor suppressor for degradation via interactions of its coiled-coil region with MDM2, the well-characterized RING E3 Ub Ligase for p53 (Wang et al., 2005). The present study shows that MAGE-C2 binds to TRIM28 directly (but not to p53), enhancing TRIM28-mediated p53 ubiquitylation, apparently independently of MDM2. Although some of the mechanistic details differ, these conclusions are

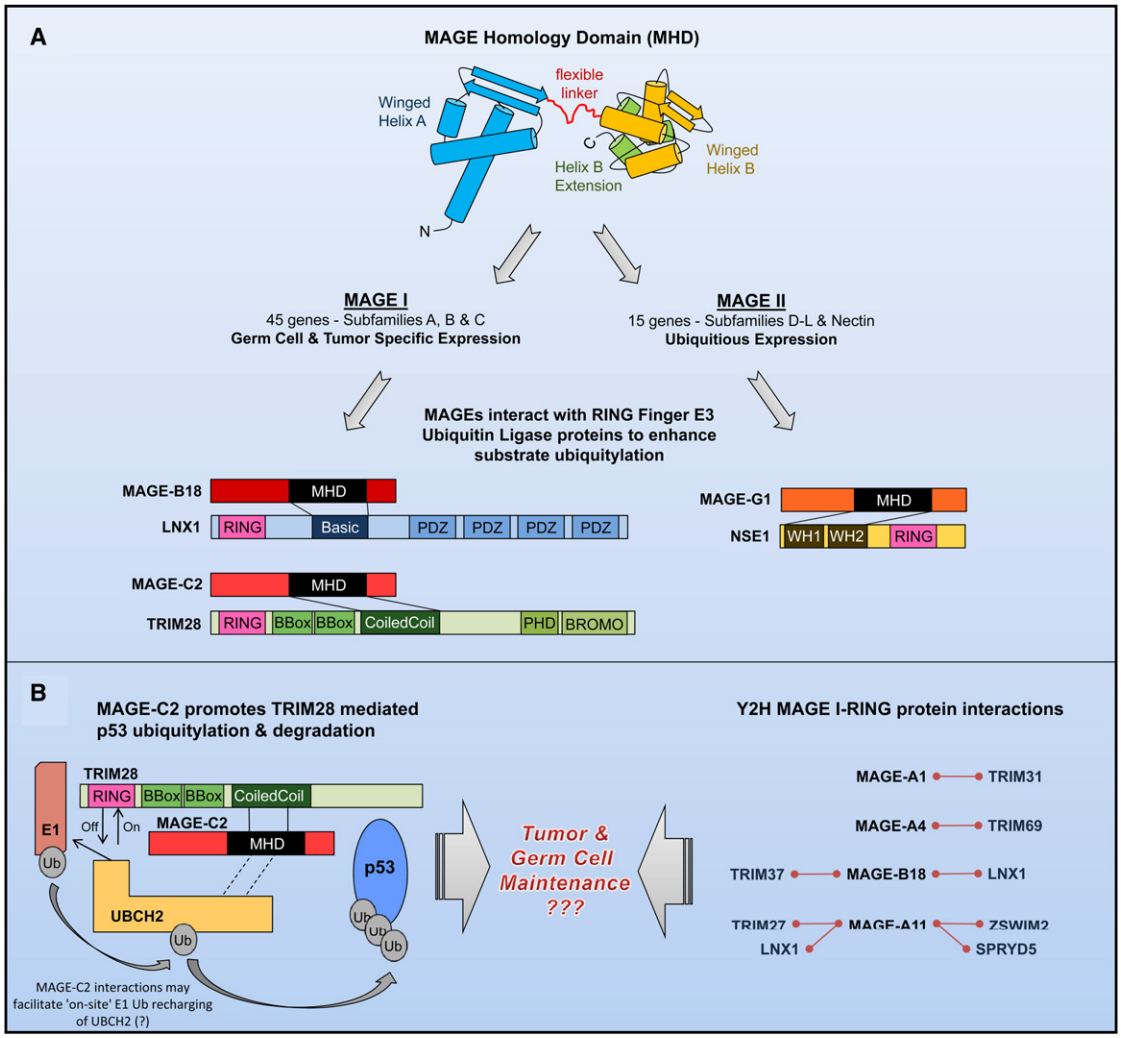

Figure 1. The Conserved MAGE I and II MHD Domains Interact with Variable Domains in RING Proteins to Promote the Ubiquitylation of Substrates, such as p53, which May Play an Important Role in Their Physiological and Pathological Functions

(A) The conserved MAGE homology domain (MHD) comprises two winged helices connected by a flexible linker region. MAGE I gene expression is normally limited to germ cells but becomes aberrantly expressed in many tumors. MAGE II genes are expressed in many different somatic tissues. A unifying theme is that MAGE I and II MHDs interact with variable domains in RING finger E3 ubiquitin ligases to form novel protein complexes that may promote ubiquitylation. (B) A model for how MAGE-C2 interactions with TRIM28 (an E3 ligase) and UbcH2 (an E2 ligase) promote the ubiquitylation of the tumor suppressor protein, p53, which may play an important physiological role in germ cell survival and pathological role in tumor survival and resistance to therapy. In addition to the MAGE I-RING interactions identified in this study, genome-wide yeast two-hybrid (Y2H) screens (Rual et al., 2005) have also identified MAGE-RING protein-protein interactions (right) that could play a novel role in regulating protein turnover to promote germ cell and tumor maintenance.

consistent with previous studies, which show that MAGE I binding to TRIM28 suppresses p53-dependent apoptosis in tumor cells (Yang et al., 2007).

An open question is how MAGE interactions at distal sites enhance RING-mediated substrate ubiquitylation. A tantalizing hint is that MAGE-C2 also binds directly to UBCH2, the same E2 ubiquitin ligase that interacts with TRIM28. It remains to be determined whether MAGE-C2's MHD domain is also required for its interaction with UBCH2. An attractive model is that a trifecta of interactions between TRIM28, UBCH2, and MAGE-C2 favors on-site recharging of UBCH2. This would potentially enable UBCH2 to dissociate from the RING and be recharged by an E1 (E2 interactions with E3s and E1s are mutually exclusive) (Deshaies and Joazeiro, 2009) while remaining stably bound to TRIM28 through interactions with MAGE-C2 (Figure 1B). Alternatively, TRIM28 may recruit two UBCH2 molecules, one via its RING domain and another via MAGE-C2, to promote the sequential assembly of a polyubiquitin chain (Deshaies and Joazeiro, 2009) on the active site of UBCH2 that is transferred en bloc to p53. This may also help to explain why the MAGE MHDs interact with RING proteins via domains other than the RING, especially if such interactions evolved subsequent to the convergence of MAGEs and their RING partners

on the same E2s. MAGE genes have also been identified in *Drosophila*, Aspergillis, and *Arabidopsis*, which could provide important insights regarding the ancestral interactions and functions of the MHD and when their interactions with RING proteins and E2s first arose.

This study fundamentally changes how we look at MAGE proteins as a whole, from curious antigens expressed on tumor cells and in the testis to novel modulators of protein homeostasis. A key question is whether MAGE I-RING interactions promote the ubiquitylation of critical substrates that confer germ cell maintenance but aberrant tumor survival. In this regard, it is interesting to note the many striking similarities that exist between germ cells and tumor cells, including their enhanced migratory and invasive properties and the ability to tolerate cyclic changes in ploidy (Simpson et al., 2005). In tumor cells, the normal complement of RING protein partners and substrates is also likely to differ, resulting in potential gain-of-function interactions between MAGEs and RINGs that may have completely unprecedented activities and pathologies. The interaction of MAGE-C2 with TRIM28 to enhance p53 degradation may also be of great clinical significance in rendering tumor cells refractory to irradiation, chemotherapy, and small molecule antagonists of MDM2.

Finally, consistent with the conclusions of Doyle et al., genome-wide yeast two-hybrid studies have also identified novel interactions between MAGEs and RING proteins (Rual et al., 2005), which will be interesting to explore in this new context (Figure 1B) . Many of the novel RING partners identified are members of the TRIM family that, similar to the MAGEs, underwent a vast expansion during mammalian evolution (Sardiello et al., 2008). It is intriguing to speculate that the expansion of both of these families of proteins and their potentially novel interactions with each other may have provided the subtleties and activities that not only make us uniquely human, but also refractory to therapy when cancer arises.

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