The specificity of *pptr-1* and B56 isoforms for Akt Thr308 but not Ser473 dephosphorylation is paralleled by recent discoveries of a PHLPP family of conserved phosphatases that target Ser473 but not Thr308 (Gao et al., 2005). As observed here for *pptr-1* in worms, different Akt family members are targeted by distinct PHLPP family members in mammalian cells (Brognard et al., 2007). Similarly, one might anticipate that multiple mammalian B56 isoforms would play a role in regulating Akt isoforms. Indeed, B56γ and B56β have previously been shown to modulate Akt signaling in different contexts (Chen et al. 2005; Rocher et al., 2007).

Notably, B56 and PHLPP isoforms are implicated in cancer and diabetes. B56γ is inactivated by SV40 small T antigen in cellular transformation (Chen et al., 2004), and B56γ is also downregulated in some tumors. Somatic mutations that result in loss of B56 binding have been reported in A subunits of PP2A, and the resulting haploinsufficiency promotes cancer (Westermarck and Hahn, 2008). Conversely, the upregulation of PHLPP1 in skeletal muscle from diabetic patients correlates with the loss of Akt-2 phosphorylation at Ser473 (Cozzone et al., 2008). Importantly, it is unlikely that Akt will be the only target of a particular B56 isoform, as p53 and Wnt signaling are both regulated by B56 isoforms. Similarly, protein kinase C signaling has been well established as a target for PHLPP beyond Akt.

Given the aforementioned complexities of different phosphatase isoforms regulating different Akt family members, it is likely that the tissue expression patterns of different mammalian B56 and PHLPP isoforms will play a major role in dictating the level of responsiveness of the PI3K-Akt pathway. Hence, inactivation of a given isoform of B56 or PHLPP might result in a restoration of insulin signaling in metabolic tissues from diabetic patients, but could trigger unrestrained proliferation in epithelial tissues. Understanding the intricacies of Akt regulation in different mammalian tissues awaits further work, but B56 subunits are now clearly front and center in the investigation of aging and disease.

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TRIM-NHL Proteins Take on miRNA Regulation

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The TRIM-NHL family of proteins is conserved among metazoans and has been shown to regulate cell proliferation and development. In this issue, Hammell et al. (2009) and Schwamborn et al. (2009) identify two members of this protein family, NHL-2 in worms and TRIM32 in mice, as positive regulators of microRNA function.

MicroRNAs (miRNAs) are small noncoding RNAs that regulate gene expression posttranscriptionally. In animals, they typically base pair imperfectly with sequences in the 3′ untranslated regions (3′UTRs) of mRNAs to either inhibit translation or accelerate mRNA decay. Following excision from longer precursors, miRNAs are loaded into ribonucleoprotein particles called miRNPs. Argonaute (AGO) and GW182 family proteins, the best-characterized components of miRNPs, act as key effectors in miRNA function. Although as many as 50% of all genes might be regulated by the hundreds of miRNAs identified in mammals, the exact mechanism of this regulation is not known. The effects of miRNAs are diverse—some targeted mRNAs undergo destabilization whereas others are mainly translationally inhibited, with the degree of repression varying for different mRNA-miRNA combinations. Consistent with this, protein cofactors

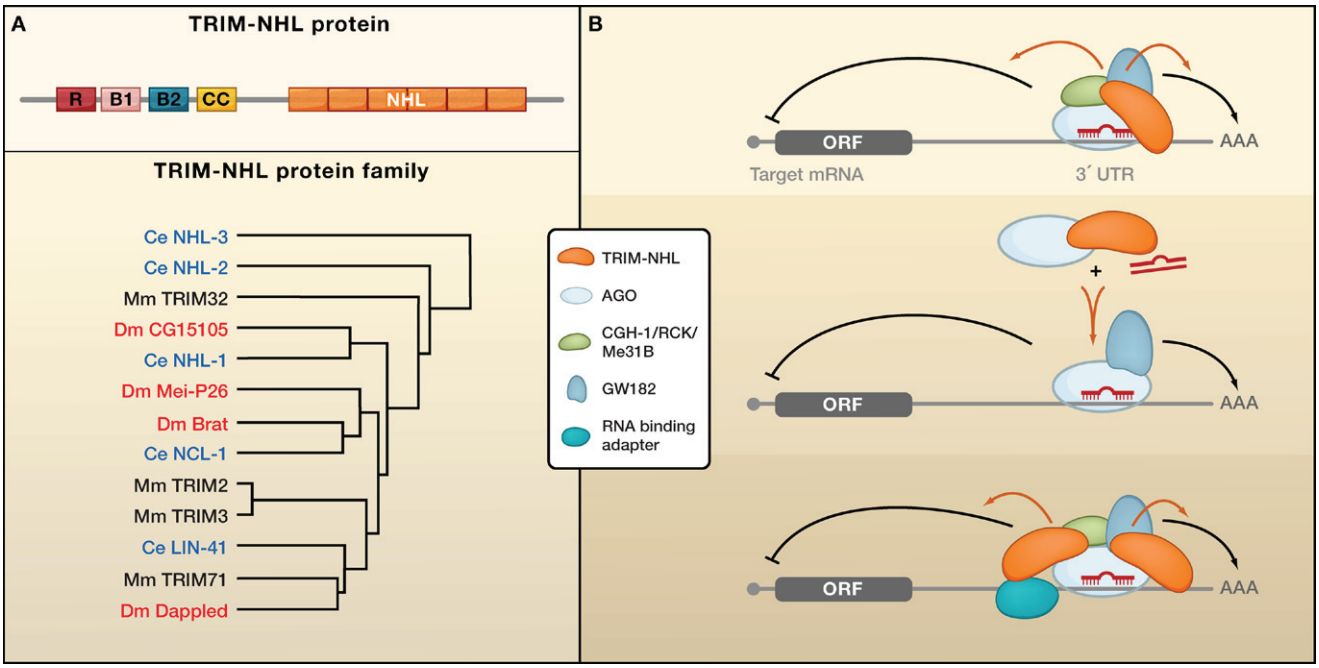

Figure 1. The TRIM-NHL Protein Family and Its Role in miRNA Regulation

(A) (Top) TRIM-NHL proteins contain the tripartite motif (TRIM), consisting of a RING domain (R), one or two B-boxes (B), a coiled-coil region (CC), and the C-terminal NHL domain composed of 4–6 NHL repeats. The RING domain confers ubiquitin ligase activity and the NHL domain mediates interaction with AGO proteins. *Drosophila melanogaster* Brat and *Caenorhabditis elegans* NCL-1 are "incomplete" TRIM-NHL proteins that lack RING domains. The TRIM motif is also found in conjunction with other types of C-terminal domains and defines a superfamily of TRIM proteins. (Bottom) Classification of mouse (black), *Drosophila* (red), and *C. elegans* (blue) TRIM-NHL proteins based on the JACOP protocol (http://myhits.isb-sib.ch/cgi-bin/jacop), a method that clusters proteins without multiple sequence alignment.

(B) Possible mechanisms of TRIM-NHL action in miRNA-mediated gene repression. (Top) TRIM-NHL proteins could increase miRNA activity by modulating the interaction of miRNP with downstream effectors, achieving miRNA specificity through recognition of the miRNA-mRNA duplex. (Middle) TRIM-NHL proteins could facilitate loading of miRNA into miRNP by contacting the miRNA duplex. (Bottom) TRIM-NHL proteins could strengthen miRNA-mRNA interactions, achieving miRNA specificity by either direct or indirect interaction with the mRNA 3′UTR at sites enriched in the vicinity of sequences recognized by specific miRNAs.

continue to be discovered that regulate the biogenesis and function of miRNAs (Ding et al., 2009; Filipowicz et al., 2008). In this issue of *Cell*, Hammell et al. (2009) and Schwamborn et al. (2009) find that two TRIM-NHL family proteins—NHL-2 in the worm *Caenorhabditis elegans* and TRIM32 in mice, respectively—positively regulate miRNA activity and play roles in developmental timing and asymmetric cell division (Figure 1).

Hammell and colleagues report that the TRIM-NHL protein NHL-2 in *C. elegans* is required for optimal *let-7* and *lsy-6* miRNA activity. Although NHL-2 deficiency results in only a relatively mild phenotype (indicating that NHL-2 is not essential for the miRNA pathway), loss of NHL-2 greatly enhances the severity of developmental defects caused by reduced levels of *let-7* or *lsy-6*. Importantly, NHL-2 affects miRNA activity without changing miRNA levels, suggesting a role for the protein in the effector phase of miRNP activity rather than in miRNA metabolism. This hypothesis is strengthened by the observations that NHL-2 interacts genetically and physically (in an RNA-dependent way) with the worm homologs of AGO and GW182 proteins. NHL-2 also interacts with the helicase CGH-1/RCK/Me31B, a component of P-bodies (cytoplasmic domains involved in the degradation and storage of repressed mRNAs) that is already implicated in miRNA repression in flies and mammals. The conclusion that TRIM-NHL proteins may enhance miRNA activity at the level of miRNP function is supported by Schwamborn et al.'s study on early neuronal development in mice. Schwamborn et al. observe that during asymmetric cell division in neural progenitor cells, the TRIM-NHL protein TRIM32 is enriched in one of the two daughter cells. The authors find that TRIM32 suppresses cell proliferation and induces neuronal differentiation. TRIM32 exerts its effect by two mechanisms: it ubiquitinates the transcription factor c-Myc, targeting it for degradation, and it enhances miRNP activity by interacting (via the NHL domain) with AGO1. The authors establish the miRNA *let-7a* as an important target of TRIM32. Consistent with this, overexpression of *let-7a* similarly promotes neuronal differentiation, whereas *let-7a* inhibition prevents it.

One of the intriguing observations made in these two reports is the apparent capacity of TRIM-NHL proteins to enhance the activity of only some miRNAs. For TRIM32, this miRNA-specialized function is supported by the specific enrichment of some miRNAs with immunoprecipitated TRIM32 (Schwamborn et al., 2009). Furthermore, Schwamborn et al. find that TRIM32 is able to stimulate repression of reporter gene expression that responds only to those enriched miRNAs. Likewise, Hammell et al. mention that *nhl-2* mutants only show genetic interactions with *let-7* and *lsy-6* mutants and not with other miRNA mutants. Several models, which

are not mutually exclusive, could explain both the miRNA-specific and the general effects of TRIM-NHL proteins (Figure 1). As mature miRNAs are very short and have their 5′ and 3′ ends anchored to specific AGO protein domains in miRNPs, there is not much room for them to interact with other proteins. Thus, TRIM-NHL proteins could recognize unique structures of the miRNA-mRNA duplex or act at an earlier step by facilitating the loading of miRNAs into miRNPs. Alternatively, TRIM-NHL proteins could interact with the mRNA 3′UTR (either directly or through adaptor proteins) and enhance miRNA-directed repression by strengthening mRNA-miRNA association. This latter model requires the enrichment of TRIM-NHL sites on the mRNA in the vicinity of binding sites for specific miRNAs. Examples of proteins binding to mRNA 3′UTRs but exerting a negative effect on miRNA repression have already been reported (Filipowicz et al., 2008).

As some TRIM-NHL proteins possess a RING domain that confers E3 ubiquitin ligase activity (Balastik et al., 2008; Kudryashova et al., 2009; Schwamborn et al., 2009), might they alter miRNP activity by ubiquitinating its components to modulate functions or for proteasomal degradation? Indeed, some GW182 proteins contain a ubiquitin-binding domain with an as yet unknown function. Though attractive, this model is not likely to apply to TRIM32 as Schwamborn et al. find that its RING domain is not required for the enhancement of miRNA-mediated repression of reporter gene expression.

Although the control of miRNA activity appears to be a general function of TRIM-NHL proteins, there may be more than one mechanism for how this control is achieved. In the fly *Drosophila melanogaster*, the TRIM-NHL proteins Brat and Mei-P26 control growth and differentiation of neuroblasts and ovarian stem cells, respectively, similar to the role of TRIM32 in mouse neuroblasts. Like TRIM32, both Brat and Mei-P26 interact with AGO1 and function posttranscriptionally to inhibit *Drosophila* Myc in differentiating daughter cells (Neumuller et al., 2008 and references therein). However, Mei-P26 appears to inhibit rather than activate the miRNA pathway. Overexpression of Mei-P26 leads to a general reduction in miRNA levels, suggesting that Mei-P26 acts at the level of miRNA biogenesis or turnover rather than at the effector step of miRNA function (Neumuller et al., 2008).

In humans, mutations in the NHL domain of TRIM32 cause a recessive hereditary muscle disorder, called limb-girdle muscular dystrophy type 2H (LGMD2H), that is phenocopied in mice lacking TRIM32. Although a neurogenic component in this disorder cannot be ruled out, these TRIM32-deficient mice do not suffer from developmental brain defects, nor do they develop brain tumors (Kudryashova et al., 2009). In contrast, when BRAT is mutated in flies, metastatic brain tumors arise. The apparent difference between these two phenotypes may be due to the redundant function in mammals of two related proteins, TRIM2 and TRIM3, which are highly expressed in the brain but not in skeletal muscle. Notably, *Trim2* knockout mice are born without any obvious brain defects but develop progressive neurodegeneration, suggesting that TRIM-NHL proteins may also serve important functions in the adult brain (Balastik et al., 2008).

Unlike their mammalian counterparts, worm TRIM-NHL proteins appear to lack functional redundancy. Indeed, Hammell et al. show that the mild phenotype caused by NHL-2 loss cannot be enhanced by the combined deletion of NHL-1, NHL-3, and NCL-1. It remains to be established whether this lack of redundancy is due to different temporal/spatial expression, different miRNA specificity, or a lack of function in the miRNA pathway for these other TRIM-NHL proteins. LIN-41 is another *C. elegans* TRIM-NHL protein with a well-documented role in developmental timing (Slack et al., 2000). Intriguingly, Hammell and colleagues observe that the precocious phenotype (premature differentiation of seam cells) caused by *lin-41* mutation is suppressed by *nhl-2* deletion, indicating antagonizing roles for these two TRIM-NHL proteins. This opposing effect might be explained, at least in part, by the fact that *lin-41* is a *let-7* target, whereas NHL-2 enhances *let-7* activity. To establish whether worm TRIM-NHL proteins other than NHL-2 are also involved in miRNA regulation, mutations in these genes should be tested for genetic interaction with miRNA factors. Indeed, LIN-41 has already been found to interact with the miRNA processing enzyme Dicer, whereas NCL-1 is known to be required for RNA interference (Duchaine et al., 2006; Kim et al., 2005).

In summary, the studies of Hammell et al. and Schwamborn et al. provide important new information about the function of TRIM-NHL proteins. These proteins emerge as key regulators of development and cell differentiation that exert their functions in many ways, including modulation of miRNA activity. It will be important to determine which of the biological effects of TRIM-NHL proteins are mediated through miRNAs and to establish their mechanism of action.

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