Gutkind, 2007), and perhaps of antitumorigenic endocannabinoids (Guzmán, 2003; Wang et al., 2008). Second, their study also unveiled that the low FFA levels and impaired growth of MAGL knocked-down cancer cells in xenografted mice can be rescued by feeding the animals a high-fat diet. What is the precise role of the MAGL-FFA connection in the crosstalk between obesity and tumorigenesis? Its possible link with dietary restriction and the oncogenic phosphatidylinositol 3-kinase pathway (Kalaany and Sabatini, 2009) could specifically be examined. Third, it was also shown that MAGL-silenced cancer cells display enhanced sensitivity to the antimigratory and antisurvival effects of an epidermal growth factor receptor tyrphostin. Could MAGL inhibitors render human tumors more responsive to the antineoplastic action of tyrosine kinase-targeted chemotherapeutic drugs? Although acute/pharmacological and sustained/genetic loss of MAGL function generated somewhat distinct metabolomic profiles in the cancer cells studied, the work by Nomura et al. (2010) turns MAGL into an unexpected and exciting new candidate for cancer therapy.

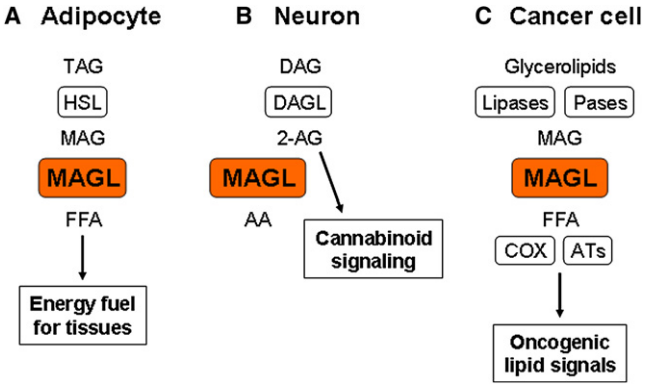


**Figure 2. The Three Ages of MAGL**

(A) MAGL catalyzes the final step of lipolysis in adipocytes, thus providing tissues with FFA fuel.

(B) MAGL deactivates 2-AG in neurons, thus tuning endocannabinoid signaling.

(C) MAGL controls FFA generation in cancer cells, thus allowing them to produce oncogenic lipid signals.

AA, arachidonic acid; AT, acyltransferase; COX, cylooxygenase; DAGL, *sn*-1 DAG lipase; Pase, lipid phosphatase. Other abbreviations are in the text.

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# Mechanism-Based Neddylation Inhibitor

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**Brownell et al. (2010) elucidate the mechanism of action of MLN4924, a NEDD8-activating enzyme inhibitor. MLN4924 requires the activity of the enzyme to generate a NEDD8-adenylate analog that potently and selectively shuts down this posttranslational modification system.**

The covalent modification of proteins with ubiquitin (Ub) and ubiquitin-like proteins (UbLs) is widely appreciated as a major eukaryotic posttranslational regulatory mechanism. To date, nine classes of UbLs with varying degrees of identity to Ub have been identified in humans (Schulman and Harper, 2009). In addition to Ub's well appreciated role in promoting protein destruction by the 26S proteasome, Ub and UbLs have numerous other functions to rapidly and conditionally alter protein function, such as changing protein localization, regulating other posttranslational modifications, and modulating protein-protein interactions.

Ub and UbLs share similar enzymatic mechanisms for their conjugation onto target proteins. Their entry into a conjugation pathway first requires ATP-dependent activation through the formation of an adenylate intermediate in the nucleotide binding pocket of their cognate E1 (activating enzyme) (Ciechanover et al., 1981; Haas et al., 1982). After this occurs, the activated molecule is transferred onto the catalytic cysteine of E1, an acceptor

sulhyrdryl, to form a high energy thiolester intermediate. The transfer of the thiolesterified molecule from an E1 to an E2 (conjugating enzyme) catalytic cysteine requires a "fully loaded" E1 complex with two bound molecules—one in its adenylate form and the other linked via a thiolester bond—with associated dramatic changes in enzyme conformation (Huang et al., 2007). The molecule is ultimately and covalently transferred when the primary amine of a lysine side chain of a protein substrate is positioned such that it destabilizes the thiolester linkage, promoting peptide bond formation. This usually involves E3s (ligases) that interact with protein substrates and activated E2s to facilitate this reaction.

Recent work by Soucy et al. (2009a) reported the characterization of MLN4924, the first cell-permeable inhibitor that selectively targets the E1 for the UbL NEDD8. The major targets for NEDD8 modification (neddylation) are cullin proteins that function as part of the catalytic core of cullin-RING ubiquitin ligases (CRLs) (Petroski and Deshaies, 2005). Reversible neddylation of cullins regulates CRL assembly and the presence of NEDD8 on CRLs enhances substrate ubiquitination by increasing the recruitment of Ub-charged E2s and promoting covalent Ub transfer. By inhibiting this E1's activity, MLN4924 effectively eviscerates downstream activities such as activation of the NEDD8 E2 UBC12 and cullin neddylation, ultimately leading to the accumulation of CRL substrates normally targeted for degradation by the 26S proteasome. As many of these substrates have critical roles regulating cell cycle progression, DNA replication, and DNA damage response mechanisms, cells treated with MLN4924 manifest hallmarks related to alterations in these and undergo apoptosis.

Remarkably, MLN4924 slows tumor growth in mouse xenograft studies and appears well tolerated at a variety of doses and treatment regimens (Soucy et al., 2009a). Given the involvement of CRLs and consequently neddylation in a myriad of processes required in all cells, it is unclear why cancer cells are more sensitive to NEDD8 E1 inhibition. Nevertheless, MLN4924 appears to have significant potential as an anticancer therapeutic and several Phase 1 clinical trials are now underway by Millennium Pharmaceuticals (Soucy et al., 2009b).

In the present work, Brownell et al. (2010) uncover a clever mechanism that underlies how MLN4924 inhibits the NEDD8 E1. MLN4924 forms a covalent adduct with the carboxy terminus of NEDD8 through its sulfamate group in a process that requires NEDD8 on the E1 active site and MLN4924 binding to the E1 nucleotide binding pocket. A hydrogen bond between oxygen atoms in MLN4924 and the backbone amide of Gly79 on the E1 β subunit lowers the pKa of the sulfamate amino group, thereby promoting its nucleophilicity. This effectively destabilizes the thiolester bond between NEDD8 and the E1 active site cysteine and leads to covalent adduct formation. Whereas the overall structure of the E1 in the presence of NEDD8, ATP, and MLN4924 appeared similar to those previously reported for it with NEDD8 and ATP (Walden et al., 2003; Huang et al., 2007), a 45° rotation of the NEDD8 G76 backbone with a 1 Å shift of the sulfamate sulfur atom on MLN4924 brings the reactants in close enough proximity to promote covalent bond formation. Experiments employing a NEDD8-MLN4924-specific antibody detected adduct accumulation with concomitant loss of neddylated cullins and accumulation of free NEDD8 after treating cells for only 5 min with the compound.

The inhibitory product generated by MLN4924 with NEDD8 resembles an Ub-adenylate analog (adenosyl-phosphoubiquitinol, APU), previously shown to inhibit the Ub E1 UBE1 (Wilkinson et al., 1990). APU binds directly to the nucleotide binding pocket to function as a competitive inhibitor with respect to ATP and a noncompetitive inhibitor with respect to Ub. ATP-PPi exchange experiments that employ E1 lacking a catalytic cysteine to isolate the initial step in NEDD8 activation determined that MLN4924 alone does not inhibit ATP binding. Thus, the initial reactions required for NEDD8 activation by E1—formation of a NEDD8-adenylate intermediate and transfer to the E1 active site—are a prerequisite for MLN4924 to bind and react with NEDD8 to form the NEDD8-MLN4924 adduct. The small size of MLN4924 presumably allows it to enter cells to rapidly react with the NEDD8 charged E1, conferring its ability to inhibit a variety of NEDD8 associated functions from E1 activation to cullin neddylation.

The similar activation mechanisms and conservation of Gly79 (described above) in all known Ub and UbL E1s suggest compounds analogous to MLN4924 should selectively modulate activities of E1s through similar mechanism-based inhibition. Such molecules would provide not only invaluable basic research tools, but may also have utility as therapeutic agents. The authors provide proof of principle through "Compound One," an analog of MLN4924 that functions as a pan inhibitor for the two Ub E1s as well as for those for NEDD8, SUMO, ISG15, and GABARAP in vitro. Thus, further efforts to fine tune various MLN4924 analogs may ultimately yield a variety of E1-selective inhibitors.

It is widely appreciated that the Ub and UbL protein modification systems have a variety of potential targets for therapeutic development efforts (Nalepa et al., 2006). To date, however, the proteasome inhibitor bortezomib (Velcade, also by Millennium Pharmaceuticals) represents the only commercially available drug directly targeting any aspect of these systems. Given the involvement of the 26S proteasome in protein homeostasis mechanisms, it is not surprising that bortezomib has toxicity issues and a variety of side effects. The ability of MLN4924 to selectively inhibit neddylation pathways, but not impact bulk protein turnover, and potently slow tumor growth in mouse xenograft studies (Soucy et al., 2009a) highlight the exciting potential of this molecule. The work by Brownell et al. (2010) provides important insight into MLN4924's mechanism of action and increases the anticipation of the forthcoming results from its clinical trials.

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# A Tale of Two Metals

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**A recent study by Thomas O'Halloran and his group at Northwestern University (Avarez et al. *Sciencexpress*, www.scienceexpress.org, 26 November 2009) has provided insights into how tetrathiomolybdate inhibits copper-trafficking proteins through metal cluster formation. The broader context of the work provides an attractive explanation of the long recognized but poorly understood antagonistic effects of Mo and Cu in living organisms.**

The relationship between copper and molybdenum as trace nutrients goes back many decades and was of particular importance to sheep farmers in Scotland, where the high molybdenum content of the soils and peats in the Scottish highlands led to severe copper deficiency in sheep. Solutions to such questions usually emerge incrementally but in their recent *Science* paper, the O'Halloran laboratory presents the results of a structural study of the interaction of tetrathiomolybdate with a copper chaperone Atx1 that provides a comprehensive explanation for many of the observed interactions between copper and molybdenum in living cells.

When tetrathiomolybdate (TM) was reacted with copper-loaded Atx1, a purple complex was formed that gave rise to crystals diffracting to 2.3 Å. Solution of the structure revealed an unusual multinuclear cluster formed by the interaction of the thio groups of TM with each of three Atx1 Cu(I) centers (Figure 1). TM did not remove copper from the chaperone as the researchers had anticipated, but rather it recruited copper-loaded chaperone molecules into an oligomeric complex. Since the overall stoichiometry was $S_6Cu_4MoS_4$, the cluster must recruit one additional copper atom, perhaps from a fourth Atx1 molecule that is converted to its apo form. They showed further that TM efficiently inhibited the transfer of copper from Atx1 to its physiological partner, the Ccc2 ATPase. TM is a promising candidate for the treatment of Wilson disease, in which copper accumulates in liver, kidney ,and brain as the result of a defect in the efflux pump ATP7B. The authors were quick to recognize that the EXAFS spectra at both the Cu and Mo K edges closely resemble those published in 2009 by Zhang et al. on complexes formed in the livers of LP rats (an animal model of Wilson disease) who had been treated with TM. The new crystal structure

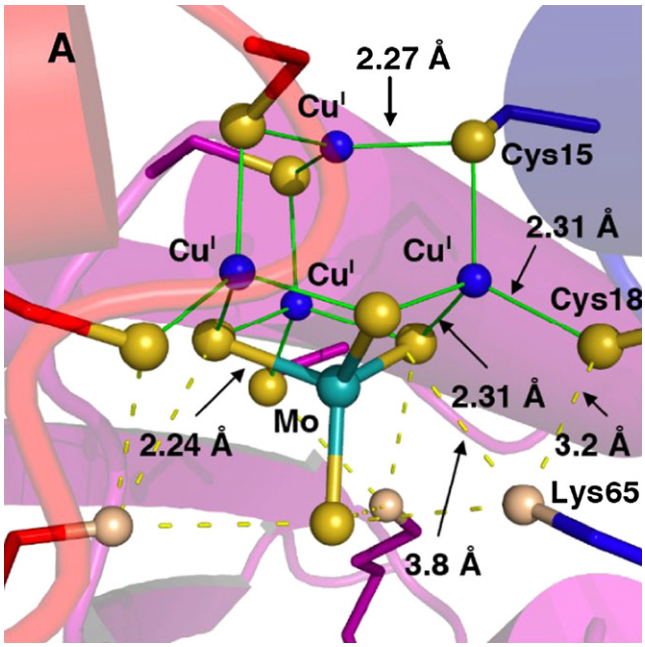


**Figure 1. Structure of the Novel $S_6Cu_4MoS_4$ Cluster Formed between Tetrathiomolybdate and Three Molecules of the Copper Chaperone Atx1**
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